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(71) Applicant: DCV, INC., doing business as BIO–TECHNICAL RESOURCES [US/US]; 1035 South Seventh Street, Manitowoc, WI 54220 (US).

(72) Inventors: BERRY, Alan; 126 Beverly Road, Bloomfield, NJ 07003 (US). RUNNING, Jeffrey, A.; 612 St. Clair Street, Manitowoc, WI 54220 (US). SEVERSON, David, K.; 1816 26th Street, Two Rivers, WI 54241 (US). BURLINGAME, Richard, P.; 808 North 9th Street, Manitowoc, WI 54220 (US).

(74) Agents: CONNELL, Gary, J. et al.; Sheridan Ross P.C., Suite 3500, 1700 Lincoln Street, Denver, CO 80203–4501 (US).

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(54) Title: VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS

(57) Abstract

A biosynthetic method for producing vitamin C (ascorbic acid, L–ascorbic acid, or AA) is disclosed. Such a method includes fermentation of a genetically modified microorganism or plant to produce L–ascorbic acid. In particular, the present invention relates to the use of microorganisms and plants having at least one genetic modification to increase the action of an enzyme involved in the ascorbic acid biosynthetic pathway. Included is the use of nucleotide sequences encoding epimerases, including the endogenous GDP–D–mannose:GDP–L–galactose epimerase from the L–ascorbic acid pathway and homologues thereof for the purposes of improving the biosynthetic production of ascorbic acid. The present invention also relates to genetically modified microorganisms, such as strains of microalgae, bacteria and yeast useful for producing L–ascorbic acid, and to genetically modified plants, useful for producing consumable plant food products.

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VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS

FIELD OF THE INVENTION

The present invention relates to vitamin C (L-ascorbic acid) production using genetically modified microorganisms and plants. In particular, the present invention relates to the use of nucleotide sugar epimerase enzymes for the biological production of ascorbic acid in plants and microorganisms.

BACKGROUND OF THE INVENTION

Nearly all forms of life, both plant and animal, either synthesize ascorbic acid (vitamin C) or require it as a nutrient. Ascorbic acid was first identified to be useful as a dietary supplement for humans and animals for the prevention of scurvy. Ascorbic acid, however, also affects human physiological functions such as the adsorption of iron, cold tolerance, the maintenance of the adrenal cortex, wound healing, the synthesis of polysaccharides and collagen, the formation of cartilage, dentine, bone and teeth, the maintenance of capillaries, and is useful as an antioxidant.

For use as a dietary supplement, ascorbic acid can be isolated from natural sources, such as rosehips, synthesized chemically through the oxidation of L-sorbose, or produced by the oxidative fermentation of calcium D-gluconate by *Acetobacter suboxidans*. Considine, "Ascorbic Acid," *Van Nostrand's Scientific Encyclopedia*, Vol. 1, pp. 237-238, (1989). Ascorbic acid (predominantly intracellular) has also been obtained through the fermentation of strains of the microalga, *Chlorella pyrenoidosa*. See U.S. Patent No. 5,001,059 by Skatrud, which is assigned to the assignee of the present application. It is believed that ascorbic acid is produced inside the chloroplasts of photosynthetic microorganisms and functions to neutralize energetic electrons produced during photosynthesis. Accordingly, ascorbic acid production is known in photosynthetic organisms as a protective mechanism.

Therefore, products and processes which improve the ability to biosynthetically produce ascorbic acid are desirable and beneficial for the improvement of human health.

SUMMARY OF THE INVENTION

One embodiment of the present invention relates to a method for producing ascorbic acid or esters thereof in a microorganism. The method includes the steps of: (a)

culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase; and (b) recovering the ascorbic acid or esters produced by the microorganism. Preferably, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In one embodiment of the method of the present invention, the microorganism further includes a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase. Such a genetic modification can include, for example, a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

In one embodiment, the genetic modification is a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, which can include GDP-D-mannose:GDP-L-galactose epimerase. In one embodiment, the epimerase binds NADPH. In one embodiment of this method, the genetic modification includes transformation of the microorganism with a recombinant nucleic acid molecule that expresses the epimerase. Such an epimerase can have a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the epimerase has a structure having an average root mean square deviation of less than about 2.5 Å, and more preferably less than about 1 Å, over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In one embodiment, the epimerase comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by

atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Such a substrate binding site preferably has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In another embodiment, the epimerase comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Such a catalytic site preferably has a tertiary structure with an average root mean square deviation of less than about 1 Å over at least about 25% of Cα positions of the tertiary structure of a catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. The catalytic site preferably includes the amino acid residues serine, tyrosine and lysine and in one embodiment, the tertiary structure positions of the amino acid residues serine, tyrosine and lysine substantially conform to tertiary structure positions of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws.

In yet another embodiment of this method, the epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50%, and in another embodiment with at least about 75%, and in yet another embodiment with at least about 90% of non-Xaa residues in SEQ ID NO:11. In another embodiment, the epimerase comprises an amino acid sequence having at least 4 contiguous amino acid residues that are 100% identical to at least 4 contiguous amino acid residues of an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10. In yet another embodiment, the recombinant nucleic acid molecule comprises a nucleic acid sequence comprising at least about 12 contiguous nucleotides having 100% identity with at least about 12

contiguous nucleotides of a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

In yet another embodiment of this method of the present invention, the epimerase comprises an amino acid sequence having a motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly. In yet another embodiment, the recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 15% identical, and in another embodiment, at least about 20% identical, and in another embodiment, at least about 25% identical, to a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

In yet another embodiment of this method of the present invention, the recombinant nucleic acid molecule comprises a nucleic acid sequence that hybridizes under stringent hybridization conditions to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase. The nucleic acid sequence encoding the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase includes nucleic acid sequences selected from the group of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5, and the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can include an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.

In one embodiment of the method of the present invention, the microorganism is selected from the group of bacteria, fungi and microalgae. In one embodiment, the microorganism is acid-tolerant. Preferred bacteria include, but are not limited to *Azotobacter* and *Pseudomonas*. Preferred fungi include, but are not limited to, yeast, including, but not limited to *Saccharomyces* yeast. Preferred microalgae include, but are not limited to, microalgae of the genera *Prototheca* and *Chlorella*, with microalgae of the genus *Prototheca* being particularly preferred.

In yet another embodiment of the method of the present invention, the microorganism is acid-tolerant and the step of culturing is conducted at a pH of less than about 6.0, and more preferably, at a pH of less than about 5.5, and even more preferably, at a pH of less than about 5.0. The step of culturing can be conducted in a fermentation medium that comprises a carbon source other than D-mannose in one embodiment, and

in another embodiment, the step of culturing is conducted in a fermentation medium that comprises glucose as a carbon source.

In yet another embodiment of the present method, the step of culturing is conducted in a fermentation medium that is magnesium (Mg) limited. Preferably, the step of culturing is conducted in a fermentation medium that is Mg limited during a cell growth phase. In one embodiment, the fermentation medium includes less than about 0.5 g/L of Mg during a cell growth phase, and more preferably, less than about 0.2 g/L of Mg during a cell growth phase, and even more preferably, less than about 0.1 g/L of Mg during a cell growth phase.

Another embodiment of the present invention relates to a microorganism for producing ascorbic acid or esters thereof. The microorganism has a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. Preferably, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase, and even more preferably, to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

In one embodiment, the microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In another embodiment, the microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a

CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11. Preferred microorganisms are disclosed as for the method discussed above.

Yet another embodiment of the present invention relates to a plant for producing ascorbic acid or esters thereof. Such a plant has a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In a preferred embodiment, the genetic modification is a genetic modification to increase the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase, and in a more preferred embodiment, the genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

In one embodiment, the plant further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase. Such a genetic modification includes a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase. Such a plant also includes a plant that has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In another embodiment, such a plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein the epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in the amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

In one embodiment, a plant for producing ascorbic acid or esters thereof according to the present invention is a microalga. Preferred microalgae include, but are not limited to microalgae of the genera *Prototheca* and *Chlorella*, with microalga of the genus *Prototheca* being particularly preferred. In another embodiment, the plant is a higher plant, with consumable higher plants being more preferred.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1A is a schematic drawing of the pathway from glucose to GDP-D-mannose in plants.

Fig. 1B is a schematic drawing of the pathway from GDP-D-mannose to L-galactose-1-phosphate in plants.

Fig. 1C is a schematic drawing of the pathway from L-galactose to L-ascorbic acid in plants.

Fig. 2A is a schematic drawing of selected carbon flow from glucose in *Prototheca*.

Fig. 2B is a schematic drawing of selected carbon flow from glucose in *Prototheca*.

Fig. 3 is a schematic drawing that shows the lineage of mutants derived from *Prototheca moriformis* ATCC 75669, and their ability to produce L-ascorbic acid.

Fig. 4 is a bar graph illustrating the conversion of substrates by resting cells of strain NA45-3 following growth in media containing various magnesium concentrations and resuspension in media containing various magnesium concentrations.

Fig. 5 is a line graph showing the relationship between specific ascorbic acid formation in cultures of *Prototheca* strains and the specific activity of GDP-D-mannose:GDP-L-galactose epimerase in extracts prepared from cells harvested from the same cultures.

Fig. 6 is a line graph showing the relationship between specific epimerase activity and the degree of magnesium limitation in two strains, ATCC 75669 and EMS13-4.

Fig. 7 depicts the overall catalytic mechanism of GDP-D-mannose:GDP-L-galactose epimerase proposed by Barber (1979, *J. Biol. Chem.* 254:7600-7603).

Fig. 8A depicts the catalytic mechanism of GDP-D-mannose-4,6-dehydratase (converts GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose).

Fig. 8B depicts the catalytic mechanism of GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (converts GDP-4-keto-6-deoxy-D-mannose to GDP-L-fucose) (Chang, et al., 1988, *J. Biol. Chem.* 263:1693-1697; Barber, 1980, *Plant Physiol.* 66:326-329).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a biosynthetic method and production microorganisms and plants for producing vitamin C (ascorbic acid, L-ascorbic acid, or AA). Such a method includes fermentation of a genetically modified microorganism to produce L-ascorbic acid. In particular, the present invention relates to the use of nucleotide sequences encoding epimerases, including the endogenous GDP-D-mannose:GDP-L-galactose epimerase from the L-ascorbic acid pathway, as well as epimerases having structural homology (e.g., by nucleotide/amino acid sequence and/or tertiary structure of the encoded protein) to GDP-4-keto-6-deoxy-D-mannose epimerase/reductases, or UDP-galactose 4-epimerases, for the purposes of improving the biosynthetic production of ascorbic acid. The present invention also relates to genetically modified microorganisms, such as strains of microalgae, bacteria and yeast useful for producing L-ascorbic acid, and to genetically modified plants, useful for producing consumable plant food products.

One embodiment of the present invention relates to a method to produce L-ascorbic acid by fermentation of a genetically modified microorganism. This method includes the steps of (a) culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase; and (b) recovering L-ascorbic acid or esters thereof. The various enzymes in this list represent the enzymes involved in the vitamin C biosynthetic pathway in plants. It is uncertain at this time

whether the enzyme represented by GDP-L-galactose phosphorylase is actually a phosphorylase or a pyrophosphorylase (i.e., GDP-L-galactose pyrophosphorylase). Therefore, use of the term "GDP-L-galactose phosphorylase" herein refers to either GDP-L-galactose phosphorylase or GDP-L-galactose pyrophosphorylase. In one aspect of the invention, this method includes the step of culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. This aspect of the present invention is discussed in detail below.

Another embodiment of the present invention relates to a genetically modified microorganism for producing L-ascorbic acid or esters thereof. Another embodiment of the present invention relates to a genetically modified plant for producing L-ascorbic acid or esters thereof. Both genetically modified microorganisms (e.g., bacteria, yeast, microalgae) and plants (e.g., higher plants, microalgae) have a genetic modification to increase the action of an enzyme selected from the group of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. In a preferred embodiment, both genetically modified microorganisms (e.g., bacteria, yeast, microalgae) and plants (e.g., higher plants, microalgae) have a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. In one embodiment, the genetic modification includes the transformation of the microorganism or plant with the epimerase as described above.

To produce significantly high yields of L-ascorbic acid by the method of the present invention, a plant and/or microorganism is genetically modified to enhance production of L-ascorbic acid. As used herein, a genetically modified plant (such as a higher plant or microalgae) or microorganism, such as a microalga (*Prototheca, Chlorella*), *Escherichia coli*, or a yeast, is modified (i.e., mutated or changed) within its genome and/or by recombinant technology (i.e., genetic engineering) from its normal (i.e., wild-type or naturally occurring) form. In a preferred embodiment, a genetically modified plant or microorganism according to the present invention has been modified by

recombinant technology. Genetic modification of a plant or microorganism can be accomplished using classical strain development and/or molecular genetic techniques, include genetic engineering techniques. Such techniques are generally disclosed herein and are additionally disclosed, for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Roessler, 1995, *Plant Lipid Metabolism*, pp. 46-48; and Roessler et al., 1994, in Bioconversion for Fuels, Himmel et al. eds., American Chemical Society, Washington D.C., pp 255-70). These references are incorporated by reference herein in their entirety.

In some embodiments, a genetically modified plant or microorganism can include a natural genetic variant as well as a plant or microorganism in which nucleic acid molecules have been inserted, deleted or modified, including by mutation of endogenous genes (e.g., by insertion, deletion, substitution, and/or inversion of nucleotides), in such a manner that the modifications provide the desired effect within the plant or microorganism. As discussed above, a genetically modified plant or microorganism includes a plant or microorganism that has been modified using recombinant technology.

As used herein, genetic modifications which result in a decrease in gene expression, an increase in inhibition of gene expression or inhibition of a gene product (i.e., the protein encoded by the gene), a decrease in the function of the gene, or a decrease in the function of the gene product can be referred to as inactivation (complete or partial), deletion, interruption, blockage, down-regulation, or decreased action of a gene. For example, a genetic modification in a gene which results in a decrease in the function of the protein encoded by such gene can be the result of a complete deletion of the gene encoding the protein (i.e., the gene does not exist, and therefore the protein does not exist), a mutation in the gene encoding the protein which results in incomplete or no translation of the protein (e.g., the protein is not expressed), or a mutation in the gene which decreases or abolishes the natural function of the protein (e.g., a protein is expressed which has decreased or no enzymatic activity).

Genetic modifications which result in an increase in gene expression or function can be referred to as amplification, overproduction, overexpression, activation, enhancement, addition, up-regulation or increased action of a gene. Additionally, a genetic modification to a gene which modifies the expression, function, or activity of the gene can

have an impact on the action of other genes and their expression products within a given metabolic pathway (e.g., by inhibition or competition). In this embodiment, the action (e.g., activity) of a particular gene and/or its product can be affected (i.e., upregulated or downregulated) by a genetic modification to another gene within the same metabolic pathway, or to a gene within a different metabolic pathway which impacts the pathway of interest by competition, inhibition, substrate formation, etc.

In general, a plant or microorganism having a genetic modification that affects L-ascorbic acid production has at least one genetic modification, as discussed above, which results in a change in the L-ascorbic acid production pathway as compared to a wild-type plant or microorganism grown or cultured under the same conditions. Such a modification in an L-ascorbic acid production pathway changes the ability of the plant or microorganism to produce L-ascorbic acid. According to the present invention, a genetically modified plant or microorganism preferably has an enhanced ability to produce L-ascorbic acid compared to a wild-type plant or microorganism cultured under the same conditions.

The present invention is based on the present inventors' discovery of the biosynthetic pathway for L-ascorbic acid (vitamin C) in plants and microorganisms. Prior to the present invention, the metabolic pathway by which plants produce L-ascorbic acid, was not completely elucidated. The present inventors have demonstrated that L-ascorbic acid production in plants, including L-ascorbic acid-producing microorganisms (e.g., microalgae), is a pathway which uses GDP-D-mannose and involves sugar phosphates and NDP-sugars. In addition, the present inventors have made the surprising discovery that both L-galactose and L-galactono-γ-lactone can be rapidly converted into L-ascorbic acid in L-ascorbic acid-producing microalgae, including *Prototheca* and *Chlorella pyrenoidosa*. The entire pathway for L-ascorbic acid production in plants is set forth in Figs. 1A-1C. More particularly, Fig. 1A shows that the production of L-ascorbic acid in plants proceeds through the production of mannose intermediates to GDP-D-mannose, followed by the conversion of GDP-D-mannose to GDP-L-galactose by GDP-D-mannose:GDP-L-galactose epimerase (also known as GDP-D-mannose-3,5-epimerase) (Fig. 1B), and then by the subsequent progression to L-galactose-1-P, L-galactose, L-galactonic acid (optional), L-galactono-γ-lactone, and L-ascorbic acid (Fig. 1C). Fig. 1B

also illustrates alternate pathways for the use of various intermediates, such as GDP-D-mannose. Certain aspects of this pathway have been independently described in a publication (Wheeler, et al., 1998, *Nature* 393:365-369), incorporated herein by reference in its entirety.

Points within the L-ascorbic acid production pathway which can be targeted by genetic modification to affect the production of L-ascorbic acid can generally be catagorized into at least one of the following pathways: (a) pathways affecting the production of GDP-D-mannose (e.g., pathways for converting a carbon source into GDP-D-mannose); (b) pathways for converting GDP-D-mannose into other compounds, (c) pathways associated with or downstream of the action of GDP-D-mannose:GDP-L-galactose epimerase, (d) pathways which compete for substrates involved in the production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid; and (e) pathways which inhibit production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid.

A genetically modified plant or microorganism useful in a method of the present invention typically has at least one genetic modification in the L-ascorbic acid production pathway which results in an enhanced production of L-ascorbic acid. In one embodiment, a genetically modified plant or microorganism has at least one genetic modification that results in: (a) an enhanced production of GDP-D-mannose; (b) an inhibition of pathways which convert GDP-D-mannose into compounds other than GDP-L-galactose; (c) an enhancement of action of the GDP-D-mannose:GDP-L-galactose epimerase; (d) an enhancement of the action of enzymes downstream of the GDP-D-mannose:GDP-L-galactose epimerase; (e) an inhibition of pathways which compete for substrates involved in the production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid; and (e) an inhibition of pathways which inhibit production of any of the intermediates within the L-ascorbic acid production pathway, and in particular, with GDP-D-mannose, GDP-L-

galactose, L-galactose-1-phosphate, L-galactose, L-galactono-γ-lactone, and/or L-ascorbic acid.

An enhanced production of GDP-D-mannose by genetic modification of the plant or microorganism can be achieved by, for example, overexpression of enzymes such as hexokinase, glucose phosphate isomerase, phosphomannose isomerase (PMI), phosphomannomutase (PMM) and/or GDP-D-mannose pyrophosphorylase (GMP). Inhibition of pathways which convert GDP-D-mannose to compounds other than GDP-L-galactose can be achieved, for example, by modifications which inhibit polysaccharide synthesis, GDP-D-rhamnose synthesis, GDP-L-fucose synthesis and/or GDP-D-mannuronic acid synthesis. An increase in the action of the GDP-D-mannose:GDP-L-galactose epimerase and of enzymes downstream of the epimerase in the L-ascorbic acid production pathway can be achieved by genetic modifications which include, but are not limited to: overexpression of the epimerase gene (i.e, by overexpression of a recombinant nucleic acid molecule encoding the epimerase gene or a homologue thereof (discussed in detail below), and/or by mutation of the endogenous or recombinant gene to enhance expression of the gene) and/or overexpression of genes downstream of the epimerase which encode subsequent enzymes in the L-ascorbic acid pathway. Finally, metabolic pathways which compete with or inhibit the L-ascorbic acid production pathway can be inhibited by deleting or mutating enzymes, substrates or products which either inhibit or compete for an enzyme, substrate or product in the L-ascorbic acid pathway.

As discussed above, a genetically modified plant or microorganism useful in the method of the present invention can have at least one genetic modification (e.g., mutation in the endogenous gene or addition of a recombinant gene) in a gene encoding an enzyme involved in the L-ascorbic acid production pathway. Such genetic modifications preferably increase (i.e., enhance) the action of such enzymes such that L-ascorbic acid is preferentially produced as compared to other possible end products in related metabolic pathways. Such genetic modifications include, but are not limited to, overexpression of the gene encoding such enzyme, and deletion, mutation, or downregulation of genes encoding competitors or inhibitors of such enzyme. Preferred enzymes for which the action of the gene encoding such enzyme can be genetically modified include: hexokinase, glucose phosphate isomerase, phosphomannose isomerase (PMI), phosphomannomutase

(PMM), GDP-D-mannose pyrophosphorylase (GMP), GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. More preferably, a genetically modified plant or microorganism useful in the present invention has a genetic modification which increases the action of an enzyme selected from the group of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and/or L-galactono-γ-lactone dehydrogenase. Even more preferably, a genetically modified plant or microorganism useful in the present invention has a genetic modification which increases the action of GDP-D-mannose:GDP-L-galactose epimerase. These enzymes and the reactions catalyzed by such enzymes are illustrated in Figs. 1A-1C.

Prior to the present invention, without knowing the L-ascorbic acid biosynthetic (i.e., production) pathway, previous mutagenesis and screening efforts were limited in that only non-lethal mutations could be detected. One embodiment of the present invention relates to elimination of a key competing enzyme that diverts carbon flow from L-ascorbic acid synthesis. If such enzyme is absolutely required for growth on glucose, then mutants lacking the enzyme (and, therefore, having increased carbon flow to L-ascorbic acid) would have been nonviable and not have been detected during prior screening efforts. One such enzyme is phosphofructokinase (PFK) (See Fig. 2A). PFK is required for growth on glucose, and is the major step drawing carbon away from L-ascorbic acid biosynthesis (Fig. 2A). Elimination of PFK would render the cells nonviable on glucose-based media. Selection of a conditional mutant where PFK was inactivated by temperature shift, for example, may allow development of a L-ascorbic acid process where cell growth is achieved under permissive fermentation conditions, and L-ascorbic acid production (from glucose) is initiated by a shift to non-permissive condition. In this example, the temperature shift would eliminate carbon flow from glucose to glycolysis via PFK, thereby shunting carbon into the L-ascorbic acid branch of metabolism. This approach has application not only in natural L-ascorbic acid producing organisms, but also in L-ascorbic acid recombinant systems (genetically engineered plant or microorganisms) as discussed herein.

Knowing the identity and mechanism of the rate-limiting pathway enzymes in the L-ascorbic acid production pathway allows for design of specific inhibitors of the enzymes that are also growth inhibitory. Selection of mutants resistant to the inhibitors allows for the isolation of strains that contain L-ascorbic acid-pathway enzymes with more favorable kinetic properties. Therefore, one embodiment of the present invention is to identify inhibitors of the enzymes that are also growth inhibitory. These inhibitors are then used to select genetic mutants that overcome this inhibition and produce L-ascorbic acid at high levels. In this embodiment, the resultant plant or microorganism is a non-recombinant strain which can then be further modified by recombinant technology, if desired. In recombinant L-ascorbic acid producing strains, random mutagenesis and screening can be used as a final step to increase L-ascorbic acid production.

In yet another embodiment genetic modifications are made to an L-ascorbic acid producing organism directly. This allows one to build upon a base of data acquired during prior classical strain improvement efforts, and perhaps more importantly, allows one to take advantage of undefined beneficial mutations that occurred during classical strain improvement. Furthermore, fewer problems are encountered when expressing native, rather than heterologous, genes. The most advanced system for development of genetic systems for microalgae has been developed for *Chlamydomonas reinhardtii*. Preferably, development of such a genetically modified production organism would include: isolation of mutant(s) with a specific nutritional requirement for use with a cloned selectable marker gene (similar to the *ura3* mutants used in yeast and fungal systems); a cloned selectable marker such as *URA3* or alternatively, identification and cloning of a gene that specifies resistance to a toxic compound (this would be analogous to the use of antibiotic resistance genes in bacterial systems, and, as is the case in yeast and other fungi, a means of inserting/removing the marker gene repeatedly would be required, unless several different selectable markers were developed); a transformation system for introducing DNA into the production organism and achieving stable transformation and expression; and, a promoter system (preferably several) for high-level expression of cloned genes in the organism.

Another embodiment of the present invention, discussed in detail below, is to place key genes or allelic variants and homologues thereof from L-ascorbic acid producing

organisms (i.e., higher plants and microalgae) into a plant or microorganism that is more amenable to molecular genetic manipulation, including endogenous L-ascorbic acid producing microorganisms and suitable plants. For example, it is possible to identify a suitable non-pathogenic organism based on the requirement of growth (on glucose) at low pH (i.e., acid-tolerant organisms, discussed in detail below).

One suitable candidate for recombinant production in any suitable host organism is the gene (nucleic acid molecule) encoding GDP-D-mannose:GDP-L-galactose epimerase and homologues of the GDP-D-mannose:GDP-L-galactose epimerase, as well as any other epimerase that has structural homology at the primary (i.e., sequence) or tertiary (i.e., three dimensional) level, to a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase, or to a UDP-galactose 4-epimerase. Many microorganisms produce GDP-D-mannose as a precursor to exopolysaccharide and glycoprotein production, even though such organisms may not make L-ascorbic acid. This aspect of the present invention is discussed in detail below.

Referring to Figs. 1A-1C, at least some of the enzymes from glucose-6-phosphate to GDP-D-mannose are present in many organisms. In fact, the entire sequence is present in bacteria such as *Azotobacter vinelandii* and *Pseudomonas aeruginosa*, and make up the early steps in the biosynthesis of the exopolysaccharide alginate. In this regard, it is possible that the only thing preventing these organisms from producing L-ascorbic acid could be the lack of GDP-D-mannose:GDP-L-galactose epimerase. The presence of PMI, PMM and GMP (see Fig. 1A) in so many organisms is important for two reasons. First, these organisms themselves could serve as alternate hosts for L-ascorbic acid production, by building on the existing early pathway enzymes and adding the required cloned genes (the epimerase and possibly others). Second, the genes encoding PMI, PMM and GMP can be cloned into a new organism where, together with the cloned epimerase, they would encode the overall pathway from glucose-6-phosphate to GDP-L- galactose.

In order to screen genomic DNA or cDNA libraries from different organisms and to isolate nucleic acid molecules encoding these enzymes such as the GDP-D-mannose:GDP-L-galactose epimerase, one can use any of a variety of standard molecular and biochemical techniques. For example, the GDP-D-mannose:GDP-L-galactose epimerase can be purified from an organism such as *Prototheca*, the N-terminal amino

acid sequence can be determined (including, if necessary, the sequence of internal peptide fragments), and this information can be used to design degenerate primers for amplifying a gene fragment from the organism's DNA. This fragment would then be used to probe the library, and subsequently fragments that hybridize to the probe would be cloned in that organism or another suitable production organism. There is ample precedent for plant enzymes being expressed in an active form in bacteria, such as *E. coli*. Alternatively, yeast are also a suitable candidate for developing a heterologous system for L-ascorbic acid production.

It is to be understood that the present invention discloses a method comprising the use of a microorganism with an ability to produce commercially useful amounts of L-ascorbic acid in a fermentation process (i.e., preferably an enhanced ability to produce L-ascorbic acid compared to a wild-type microorganism cultured under the same conditions). This method is achieved by the genetic modification of one or more genes encoding a protein involved in an L-ascorbic acid pathway which results in the production (expression) of a protein having an altered (e.g., increased or decreased) function as compared to the corresponding wild-type protein. Preferably, such genetic modification is achieved by recombinant technology. It will be appreciated by those of skill in the art that production of genetically modified plants or microorganisms having a particular altered function as described elsewhere herein (e.g., an enhanced ability to produce GDP-D-mannose:GDP-L-galactose epimerase), such as by transformation of the plant or microorganism with a nucleic acid molecule which encodes a particular enzyme, can produce many organisms meeting the given functional requirement, albeit by virtue of a variety of different genetic modifications. For example, different random nucleotide deletions and/or substitutions in a given nucleic acid sequence may all give rise to the same phenotypic result (e.g., decreased enzymatic activity of the protein encoded by the sequence). The present invention contemplates any such genetic modification which results in the production of a plant or microorganism having the characteristics set forth herein.

A microorganism to be used in the fermentation method of the present invention is preferably a bacterium, a fungus, or a microalga which has been genetically modified according to the disclosure above. More preferably, a microorganism useful in the present

invention is a microalga which is capable of producing L-ascorbic acid, although the present invention includes microorganisms which are genetically engineered to produce L-ascorbic acid using the knowledge of the key components of the pathway and the guidance provided herein. Even more preferably, a microorganism useful in the present invention is an acid-tolerant microorganism, such as microalgae of the genera *Prototheca* and *Chlorella*. Acid-tolerant yeast and bacteria are also known in the art. Acid-tolerant microorganisms are discussed in detail below. Particularly preferred microalgae include microalgae of the genera, *Prototheca* and *Chlorella*, with *Prototheca* being most preferred. All known species of *Prototheca* produce L-ascorbic acid. Production of ascorbic acid by microalgae of the genera *Prototheca* and *Chlorella* is described in detail in U.S. Patent No. 5,792,631, issued August 11, 1998, and in U.S. Patent No. 5,900,370, issued May 4, 1999, both of which are incorporated herein by reference in their entirety. Preferred bacteria for use in the present invention include, but are not limited to, *Azotobacter*, *Pseudomonas*, and *Escherichia*, although acid-tolerant bacteria are more preferred. Preferred fungi for use in the present invention include yeast, and more preferably, yeast of the genus, *Saccharomyces*. A microorganism for use in the fermentation method of the present invention can also be referred to as a production organism. According to the present invention, microalgae can be referred to herein either as microorganisms or as plants.

A preferred plant to genetically modify according to the present invention is preferably a plant suitable for consumption by animals, including humans. More preferably, such a plant is a plant that naturally produces L-ascorbic acid, although other plants can be genetically modified to produce L-ascorbic acid using the guidance provided herein.

The L-ascorbic acid production pathways of the microalgae *Prototheca* and *Chlorella pyrenoidosa* will be addressed as specific embodiments of the present invention are described below. It will be appreciated that other plants and, in particular, other microorganisms, have similar L-ascorbic acid pathways and genes and proteins having similar structure and function within such pathways. It will also be appreciated that plants and microorganisms which do not naturally produce L-ascorbic acid can be modified according to the present invention to produce L-ascorbic acid. As such, the principles

discussed below with regard to *Prototheca* and *Chlorella pyrenoidosa* are applicable to other plants and microorganisms, including genetically modified plants and microorganisms.

In one embodiment of the present invention, the action of an enzyme in the L-ascorbic acid production pathway is increased by amplification of the expression (i.e., overexpression) of an enzyme in the pathway, and particularly, the GDP-D-mannose:GDP-L-galactose epimerase, homologues of the epimerase, and/or enzymes downstream of the epimerase. Overexpression of an enzyme can be accomplished, for example, by introduction of a recombinant nucleic acid molecule encoding the enzyme. It is preferred that the gene encoding an enzyme in the L-ascorbic acid production pathway be cloned under control of an artificial promoter. The promoter can be any suitable promoter that will provide a level of enzyme expression required to maintain a sufficient level of L-ascorbic acid in the production organism. Preferred promoters are constitutive (rather than inducible) promoters, since the need for addition of expensive inducers is therefore obviated. The gene dosage (copy number) of a recombinant nucleic acid molecule according to the present invention can be varied according to the requirements for maximum product formation. In one embodiment, the recombinant nucleic acid molecule encoding a gene in the L-ascorbic acid production pathway is integrated into the chromosomes of the microorganism.

It is another embodiment of the present invention to provide a microorganism having one or more enzymes in the L-ascorbic acid production pathway with improved affinity for its substrates. An enzyme with improved affinity for its substrates can be produced by any suitable method of genetic modification or protein engineering. For example, computer-based protein engineering can be used to design an epimerase protein with greater stability and better affinity for its substrate. See for example, Maulik et al., 1997, *Molecular Biotechnology: Therapeutic Applications and Strategies*, Wiley-Liss, Inc., which is incorporated herein by reference in its entirety.

Recombinant nucleic acid molecules encoding proteins in the L-ascorbic acid production pathway can be modified to enhance or reduce the function (i.e., activity) of the protein, as desired to increase L-ascorbic acid production, by any suitable method of genetic modification. For example, a recombinant nucleic acid molecule encoding an

enzyme can be modified by any method for inserting, deleting, and/or substituting nucleotides, such as by error-prone PCR. In this method, the gene is amplified under conditions that lead to a high frequency of misincorporation errors by the DNA polymerase used for the amplification. As a result, a high frequency of mutations are obtained in the PCR products. The resulting gene mutants can then be screened for enhanced substrate affinity, enhanced enzymatic activity, or reduced/increased inhibitory ability by testing the mutant genes for the ability to confer increased L-ascorbic acid production onto a test microorganism, as compared to a microorganism carrying the non-mutated recombinant nucleic acid molecule.

Another embodiment of the present invention includes a microorganism in which competitive side reactions are blocked, including all reactions for which GDP-D-mannose is a substrate other than the production of L-ascorbic acid. In a preferred embodiment, a microorganism having complete or partial inactivation (decrease in the action of) of genes encoding enzymes which compete with the GDP-D-mannose:GDP-L-galactose epimerase for the GDP-D-mannose substrate is provided. Such enzymes include GDP-D-mannase and/or GDP-D-mannose-dehydrogenase. As used herein, inactivation of a gene can refer to any modification of a gene which results in a decrease in the activity (i.e., expression or function) of such a gene, including attenuation of activity or complete deletion of activity.

As discussed above, a particularly preferred aspect of the method to produce L-ascorbic acid by fermentation of a genetically modified microorganism of the present invention includes the step of culturing in a fermentation medium a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. According to the present invention, such an epimerase can include the endogenous GDP-D-mannose:GDP-L-galactose epimerase from the L-ascorbic acid pathway, described above, as well as any other epimerase that has structural homology at the primary (i.e., sequence) or tertiary (i.e., three dimensional) level, to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, or to a UDP-galactose 4-epimerase. Such structural homology is discussed in detail below. Preferably, such an epimerase is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. In one embodiment, the genetic modification includes transformation of the

microorganism with a recombinant nucleic acid molecule that expresses such an epimerase.

Therefore, the epimerase encompassed in the method and organisms of the present invention includes the endogenous epimerase which operates in the naturally occurring ascorbic acid biosynthetic pathway (referred to herein as GDP-D-mannose:GDP-L-galactose epimerase), GDP-4-keto-6-deoxy-D-mannose epimerase/ reductases, and any other epimerase which is capable of catalyzing the conversion of GDP-D mannose to GDP-L-galactose and which is structurally homologous to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase. An epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose according the present invention can be identified by biochemical and functional characteristics as well as structural characteristics. For example, an epimerase according to the present invention is capable of acting on GDP-D-mannose as a substrate, and more particularly, such an epimerase is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. It is to be understood that such capabilities need not necessarily be the normal or natural function of the epimerase as it acts in its endogenous (i.e., natural) environment. For example, GDP-4-keto-6-deoxy-D-mannose epimerase/reductase in its natural environment under normal conditions, catalyzes the conversion of GDP-D-mannose to GDP-L-fucose and does not act directly on GDP-D-mannose (See Fig.8A, B), however, such an epimerase is encompassed by the present invention for use in catalyzing the conversion of GDP-D-mannose to GDP-L-galactose for production of ascorbic acid, to the extent that it is capable of, or can be modified to be capable of, catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. Therefore, the present invention includes epimerases which have the desired enzyme activity for use in production of ascorbic acid, are capable of having such desired enzyme activity, and/or are capable of being modified or induced to have such desired enzyme activity.

In one embodiment, an epimerase according to the present invention includes an epimerase that catalyzes the reaction depicted in Fig. 7. In another embodiment, an epimerase according to the present invention includes an epimerase that catalyzes the first of the reactions depicted in Fig. 8B. In one embodiment, an epimerase according to the

present invention binds to NADPH. In another embodiment, an epimerase according to the present invention is NADPH-dependent for enzyme activity.

As discussed above, the present inventors have discovered that a key enzyme in L-ascorbic acid biosynthesis in plants and microorganisms is GDP-D-mannose:GDP-L-galactose epimerase (refer to Figs. 1A-1C). One embodiment of the invention described herein is directed to the manipulation of this enzyme and structural homologues of this enzyme to increase L-ascorbic acid production in genetically engineered plants and/or microorganisms. More particularly, the GDP-D-mannose:GDP-L-galactose epimerase of the L-ascorbic acid pathway and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases are believed to be structurally homologous at both the sequence and tertiary structure level; a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase is believed to be capable of functioning in the L-ascorbic acid biosynthetic pathway; and a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or homologue thereof may be superior to a GDP-D-mannose-GDP-L-galactose epimerase for increasing L-ascorbic acid production in genetically engineered plants and/or microorganisms. Furthermore, the present inventors disclose the use of a nucleotide sequence encoding all or part of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase as a probe to identify the gene encoding GDP-D-mannose:GDP-L-galactose epimerase. Similarly, the present inventors disclose the use of a nucleotide sequence of the gene encoding GDP-4-keto-6-deoxy-D-mannose epimerase/reductase to design oligonucleotide primers for use in a PCR-based strategy for identifying and cloning a gene encoding GDP-D-mannose:GDP-L-galactose epimerase.

Without being bound by theory, the present inventors believe that the following evidence supports the novel concept that the GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases have significant structural homology at the level of sequence and/or tertiary structure, and that the GDP-4-keto-6-deoxy-D-mannose epimerase/reductases and/or homologues thereof would be useful for production of ascorbic acid and/or for isolating the endogenous GDP-D-mannose:GDP-L-galactose epimerase.

Although prior to the present invention, it was not known that the GDP-D-mannose:GDP-L-galactose epimerase enzyme (also known as GDP-D-mannose-3,5-epimerase) plays a critical role in L-ascorbic acid biosynthesis, this enzyme was previously

described to catalyze the overall reversible reaction between GDP-D-mannose and GDP-L-galactose (Barber, 1971, *Arch. Biochem. Biophys.* 147:619-623; Barber, 1975, *Arch. Biochem. Biophys.* 167:718-722; Barber, 1979, *J. Biol. Chem.* 254:7600-7603; Hebda, et al., 1979, *Arch. Biochem. Biophys.* 194:496-502; Barber and Hebda, 1982, *Meth. Enzymol.*, 83:522-525). Despite these studies, GDP-D-mannose:GDP-L-galactose epimerase has never been well characterized nor has the gene encoding this enzyme been cloned and sequenced. Since the original work by Barber, GDP-D-mannose:GDP-L-galactose epimerase activity has been detected in the colorless microalga *Prototheca moriformis* by the assignee of the present application, and in *Arabidopsis thaliana* and pea embryonic axes (Wheeler, et al., 1998, *ibid.*).

Barber (1979, *J. Biol. Chem.* 254:7600-7603) proposed a mechanism for GDP-D-mannose:GDP-L-galactose epimerase partially purified from the green microalga *Chlorella pyrenoidosa*. The overall conversion of GDP-D-mannose to GDP-L-galactose was proposed to proceed by oxidation of the hexosyl moiety at C-4 to a keto intermediate, ene-diol formation, and inversion of the configurations at C-3 and C-5 upon rehydration of the double bonds and stereospecific reduction of the keto group. The proposed mechanism is depicted in Fig. 7.

Based on Barber's work, Feingold and Avigad (1980, In *The Biochemistry of Plants*, Vol. 3: Carbohydrates; Structure and Function, P.K. Stompf and E.E. Conn, eds., Academic Press, NY) elaborated further on the proposed mechanism for GDP-D-mannose:GDP-L-galactose epimerase. This mechanism is based on the assumption that the epimerase contains tightly bound NAD^+, and transfer of a hydride ion from C-4 of the substrate (GDP-D-mannose) to enzyme-associated NAD^+ converts the enzyme to the reduced (NADH)form, generating enzyme-bound GDP-4-keto-D-mannose. The latter would then undergo epimerization by an ene-diol mechanism. The final product (GDP-L-galactose) would be released from the enzyme after stereospecific transfer of the hydride ion originally removed from C-4, simultaneously regenerating the oxidized form of the enzyme.

L-fucose (6-deoxy-L-galactose) is a component of bacterial lipopolysaccharides, mammalian and plant glycoproteins and polysaccharides of plant cell walls. L-fucose is synthesized *de novo* from GDP-D-mannose by the sequential action of GDP-D-mannose-

4,6-dehydratase (an NAD(P)-dependent enzyme), and a bifunctional GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (NADPH-dependent), also referred to in scientific literature as GDP-fucose synthetase (Rizzi, et al., 1998, *Structure* 6:1453-1465; Somers, et al., 1998, *Structure* 6:1601-1612). This pathway for L-fucose biosynthesis appears to be ubiquitous (Rizzi, et al., 1998, *Structure* 6:1453-1465). The mechanisms for GDP-D-mannose-4,6-dehydratase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase are shown in Fig. 8A, B (Chang, et al., 1988, *J. Biol. Chem.* 263:1693-1697; Barber, 1980, *Plant Physiol.* 66:326-329).

Comparison of Figs. 7 and 8A, B reveals that Barber's proposed mechanism for GDP-D-mannose:GDP-L-galactose epimerase is analogous to the reaction mechanism for GDP-4-keto-6-deoxy-D-mannose epimerase/reductase. The same mechanism has also been demonstrated for the epimerization reaction that occurs in the biosynthesis of two TDP-6-deoxy hexoses, TDP-L-rhamnose and TDP-6-deoxy-L-talose, from TDP-D-glucose (Liu and Thorson, 1994, *Ann. Rev. Microbiol.* 48:223-256). In the latter cases, however, the final reduction at C-4 is catalyzed by NADPH-dependent reductases that are separate from the epimerase enzyme. These reductases have opposite stereospecificity, providing either TDP-L-rhamnose or TDP-6-deoxy-L-talose (Liu and Thorson, 1994, *Ann. Rev. Microbiol.* 48:223-256).

In all of the mechanisms described above, NAD(P)H is required for the final reduction at C-4 (refer to Fig. 8B). In the work of Hebda, et al. (1979, *Arch. Biochem. Biophys.* 194:496-502), it was reported that GDP-D-mannose:GDP-L-galactose epimerase from *C. pyrenoidosa* did not require NAD, NADP or NADH for activity. Strangely, NADPH was not tested. Based on the analogous mechanisms shown in Figs. 7 and 8A, B, the present inventors believe that it is likely that GDP-D-mannose:GDP-L-galactose epimerase from *C. pyrenoidosa* requires NADPH for the final reduction step. Why activity was detected *in vitro* without NADPH addition is not known, but tight *binding of NADPH to the enzyme could explain this observation. On the other hand, if the proposed mechanism of Feingold and Avigad (1980, in *The Biochemistry of Plants*, Vol. 3, p. 101-170: Carbohydrates; Structure and Function, P.K. Stompf and E.E. Conn, ed., Academic Press, NY) is correct, the reduced enzyme-bound cofactor generated in the first oxidation step of the epimerase reaction would serve as the source of electrons for

the final reduction of the keto group at C-4 back to the alcohol. Thus no addition of exogenous reduced cofactor would be required for activity *in vitro*.

Recently, a human gene encoding the bifunctional GDP-4-keto-6-deoxy-D-mannose epimerase/reductase was cloned and sequenced (Tonetti, et al., 1996, *J. Biol. Chem.* 271-27274-27279). This amino acid sequence of the human GDP-4-keto-6-deoxy-D-mannose epimerase/reductase shows significant homology (29% identity) to the *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (Tonetti, et al., 1998, *Acta Cryst.* D54:684-686; Somers, et al., 1998, *Structure* 6:1601-1612, both of which are incorporated herein by reference in their entireties). Tonetti et al. and Somers et al. additionally disclosed the tertiary (three dimensional) structure of the *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (also known as GDP-fucose synthetase), and noted significant structural homology with another epimerase, UDP-galactose 4-epimerase (GalE). These epimerases also share significant homology at the sequence level. Since no gene encoding a GDP-D-mannose:GDP-L-galactose epimerase has been cloned and sequenced, homology with genes encoding GDP-4-keto-6-deoxy-D-mannose epimerase/reductases or with genes encoding a UDP-galactose 4-epimerase has not been demonstrated. However, based on the similarity of the reaction products for GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (i.e., GDP-L-galactose and GDP-6-deoxy-L-galactose [i.e., GDP-L-fucose], respectively) and the common catalytic mechanisms (Figs. 7 and 8A, B) the present inventors believe that the genes encoding the enzymes will have a high degree of sequence homology, as well as tertiary structural homology.

Significant structural homology between GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductases may allow a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, or a homologue thereof, to function in the L-ascorbic acid biosynthetic pathway, and a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase could potentially be even better than a GDP-D-mannose-GDP-L-galactose epimerase for increasing L-ascorbic acid production in genetically engineered plants and/or microorganisms. Furthermore, a nucleotide sequence encoding all or part of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can be used as a probe to identify the gene encoding GDP-D-mannose:GDP-L-galactose epimerase. Likewise, the

nucleotide sequence of the gene encoding GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase can be used to design oligonucleotide primers for use in a PCR-based strategy for identifying and cloning a gene encoding GDP-D-mannose:GDP-L-galactose epimerase.

The ability to substitute GDP-4-keto-6-D-mannose epimerase/reductase for GDP-D-mannose:GDP-L-galactose epimerase to enhance L-ascorbic acid biosynthesis in plants or microorganisms depends on the ability of GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase to act directly on GDP-D-mannose to form GDP-L-galactose. Evidence supporting this possibility already exists. *Arabidopsis thaliana murl* mutants are defective in GDP-D-mannose-4,6-dehydratase activity (Bonin, et al., 1997, *Proc. Natl. Acad. Sci.* 94:2085-2090). These mutants are thus blocked in GDP-L-fucose biosynthesis, and consequently have less than 2% of the normal amounts of L-fucose in the primary cell walls of aerial portions of the plant (Zablackis, et al., 1996, *Science* 272:1808-1810). The *murl* mutants are more brittle than wild-type plants, are slightly dwarfed and have an apparently normal life cycle (Zablackis, et al., 272:1808-1810). When *murl* mutants are grown in the presence of exogenous L-fucose, the L-fucose content in the plant is restored to the wild-type state (Bonin, et al., 1997, *Proc. Natl. Acad. Sci.* 94:2085-2090). It was discovered (Zablackis, et al., 1996, *Science* 272:1808-1810) that *murl* mutants contain, in the hemicellulose xyloglucan component of the primary cell wall, L-galactose in place of the normal L-fucose. L-galactose is not normally found in the xyloglucan component, but in *murl* mutants L-galactose partly replaces the terminal L-fucosyl residue. Bonin, et al. (1997, *Proc. Natl. Acad. Sci.* 94:2085-2090) hypothesized that in the absence of a functional GDP-D-mannose-4,6-dehydratase in the *murl* mutants, the GDP-4-keto-6-deoxy-D-mannose epimerase/reductase normally involved in L-fucose synthesis may be able to use GDP-D-mannose directly, forming GDP-L-galactose. Another possibility, however, is that the enzymes involved in L-ascorbic acid biosynthesis in *A. thaliana* are responsible for forming GDP-L-galactose in the *murl* mutant. If this were true, it would suggest that in the wild-type plant, some mechanism exists that prevents GDP-L-galactose formed in the L-ascorbic acid pathway from entering cell wall biosynthesis and substituting for (competing with) GDP-L-fucose for incorporation into the xyloglucan

component (since L-galactose is not present in the primary cell wall of the wild-type plant).

Because of the similar reaction mechanisms of GDP-D-mannose:GDP-L-galactose epimerase and GDP-4-keto-6-deoxy-D-mannose epimerase/reductase, and because of the evidence that GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can act directly on GDP-D-mannose to form GDP-L-galactose, the present inventors believe that genes encoding all epimerases and epimerase/reductases that act on GDP-D-mannose have high homology. As such, one aspect of the present invention relates to the use of any epimerase (and nucleic acid sequences encoding such epimerase) having significant homology (at the primary, secondary and/or tertiary structure level) to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or to a UDP-galactose 4-epimerase for the purpose of improving the biosynthetic production of L-ascorbic acid.

Therefore, as described above, one embodiment of the present invention relates to a method for producing ascorbic acid or esters thereof in a microorganism, which includes culturing a microorganism having a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. Also included in the present invention are genetically modified microorganisms and plants in which the genetic modification increases the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose.

According to the present invention, an increase in the action of the GDP-D-mannose:GDP-L-galactose epimerase in the L-ascorbic acid production pathway can be achieved by genetic modifications which include, but are not limited to overexpression of the GDP-D-mannose:GDP-L-galactose epimerase gene, a homologue of such gene, or of any recombinant nucleic acid sequence encoding an epimerase that is homologous in primary (nucleic acid or amino acid sequence) or tertiary (three dimensional protein) structure to a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, such as by overexpression of a recombinant nucleic acid molecule encoding the epimerase gene or a homologue thereof, and/or by mutation of the endogenous or recombinant gene to enhance expression of the gene.

According to the present invention, an epimerase that has a tertiary structure that is homologous to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/

reductase is an epimerase that has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws (Table 12). In another embodiment, an epimerase that has a tertiary structure that is homologous to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase is an epimerase that has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS. As used herein, a "tertiary structure" or "three dimensional structure" of a protein, such terms being interchangeable, refers to the components and the manner of arrangement of the components in three dimensional space to constitute the protein. The use of the term "substantially conforms" refers to at least a portion of a tertiary structure of an epimerase which is sufficiently spatially similar to at least a portion of a specified three dimensional configuration of a particular set of atomic coordinates (e.g., those represented by Brookhaven Protein Data Bank Accession Code 1bws) to allow the tertiary structure of at least said portion of the epimerase to be modeled or calculated (i.e., by molecular replacement) using the particular set of atomic coordinates as a basis for estimating the atomic coordinates defining the three dimensional configuration of the epimerase.

More particularly, a tertiary structure that substantially conforms to a given set of atomic coordinates is a structure having an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, a structure that substantially conforms to a given set of atomic coordinates is a structure wherein such structure has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, such structure has the

recited average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, such structure has the recited average root-mean-square deviation (RMSD) value over about 100% of the Cα positions as compared to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Methods to calculate RMSD values are well known in the art. Various software programs for determining the tertiary structural homology between one or more proteins are known in the art and are publicly available, such as QUANTA (Molecular Simulations Inc.).

A preferred epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose according to the method and genetically modified organisms of the present invention includes an epimerase that comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the tertiary structure of the substrate binding site of the epimerase has an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions

as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the substrate binding site of the epimerase has the recited average root-mean-square deviation (RMSD) value over about 100% of the Cα positions as compared to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. The tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws is discussed in detail in Rizzi et al., 1998, *ibid.* Additionally, the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS is discussed in detail in Somers et al., 1998, *ibid.*

Another preferred epimerase according to the present invention includes an epimerase that comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. Preferably, the tertiary structure of the catalytic site of the epimerase has an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å, over at least about 25% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws. In other embodiments, the tertiary structure of the catalytic site of the epimerase has the recited average root-mean-square deviation (RMSD) value over at least about 50% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the catalytic site of the epimerase has the recited

average root-mean-square deviation (RMSD) value over at least about 75% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws, and in another embodiment, the tertiary structure of the catalytic site of the epimerase has the recited average root-mean-square deviation (RMSD) value over 100% of the Cα positions as compared to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

In one embodiment, an epimerase encompassed by the present invention includes an epimerase that has a catalytic site which includes amino acid residues: serine, tyrosine and lysine. In a preferred embodiment, the tertiary structure positions of the amino acid residues serine, tyrosine and lysine substantially conform to the tertiary structure position of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws. The tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws is discussed in detail in Rizzi et al., 1998, *ibid.* Additionally, the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by the atomic coordinates having Brookhaven Protein Data Bank Accession Code 1GFS is discussed in detail in Somers et al., 1998, *ibid.*

In an even more preferred embodiment, the above definition of "substantially conforms" can be further defined to include atoms of amino acid side chains. As used herein, the phrase "common amino acid side chains" refers to amino acid side chains that are common to both the structures which substantially conforms to a given set of atomic coordinates and the structure that is actually represented by such atomic coordinates. Preferably, a tertiary structure that substantially conforms to a given set of atomic coordinates is a structure having an average root-mean-square deviation (RMSD) of less than about 2.5 Å, and more preferably, less than about 2 Å, and, in increasing preference, less than about 1.5 Å, less than about 1 Å, less than about 0.5 Å, and most preferably, less than about 0.3 Å over at least about 25% of the common amino acid side chains as

compared to the tertiary structure represented by the given set of atomic coordinates. In another embodiment, a structure that substantially conforms to a given set of atomic coordinates is a structure having the recited average root-mean-square deviation (RMSD) value over at least about 50% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates, and in another embodiment, such structure has the recited average root-mean-square deviation (RMSD) value over at least about 75% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates, and in another embodiment, such a structure has the recited average root-mean-square deviation (RMSD) value over 100% of the common amino acid side chains as compared to the tertiary structure represented by the given set of atomic coordinates.

A tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can be modeled by a suitable modeling computer program such as MODELER (A. Sali and T.L. Blundell, *J. Mol. Biol.*, vol. 234:779-815, 1993 as implemented in the Insight II Homology software package (Insight II (97.0), MSI, San Diego)), using information, for example, derived from the following data: (1) the amino acid sequence of the epimerase; (2) the amino acid sequence of the related portion(s) of the protein represented by the specified set of atomic coordinates having a three dimensional configuration; and, (3) the atomic coordinates of the specified three dimensional configuration. Alternatively, a tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can be modeled using data generated from analysis of a crystallized structure of the epimerase. A tertiary structure of an epimerase which substantially conforms to a specified set of atomic coordinates can also be calculated by a method such as molecular replacement. Methods of molecular replacement are generally known by those of skill in the art (generally described in Brunger, *Meth. Enzym.*, vol. 276, pp. 558-580, 1997; Navaza and Saludjian, *Meth. Enzym.*, vol. 276, pp. 581-594, 1997; Tong and Rossmann, *Meth. Enzym.*, vol. 276, pp. 594-611, 1997; and Bentley, *Meth. Enzym.*, vol. 276, pp. 611-619, 1997, each of which are incorporated by this reference herein in their entirety) and are performed in a software program including, for example, XPLOR (Brunger, et al., *Science*, vol. 235, p. 458, 1987). In addition, a structure can be modeled using techniques generally described by,

for example, Sali, *Current Opinions in Biotechnology*, vol. 6, pp. 437-451, 1995, and algorithms can be implemented in program packages such as Homology 95.0 (in the program Insight II, available from Biosym/MSI, San Diego, CA). Use of Homology 95.0 requires an alignment of an amino acid sequence of a known structure having a known three dimensional structure with an amino acid sequence of a target structure to be modeled. The alignment can be a pairwise alignment or a multiple sequence alignment including other related sequences (for example, using the method generally described by Rost, *Meth. Enzymol.*, vol. 266, pp. 525-539, 1996) to improve accuracy. Structurally conserved regions can be identified by comparing related structural features, or by examining the degree of sequence homology between the known structure and the target structure. Certain coordinates for the target structure are assigned using known structures from the known structure. Coordinates for other regions of the target structure can be generated from fragments obtained from known structures such as those found in the Protein Data Bank maintained by Brookhaven National Laboratory, Upton, NY. Conformation of side chains of the target structure can be assigned with reference to what is sterically allowable and using a library of rotamers and their frequency of occurrence (as generally described in Ponder and Richards, *J. Mol. Biol.*, vol. 193, pp. 775-791, 1987). The resulting model of the target structure, can be refined by molecular mechanics (such as embodied in the program Discover, available from Biosym/MSI) to ensure that the model is chemically and conformationally reasonable.

According to the present invention, an epimerase that has a nucleic acid sequence that is homologous at the primary structure level (i.e., is a homologue of) to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase includes any epimerase encoded by a nucleic acid sequence that is at least about 15%, and preferably at least about 20%, and more preferably at least about 25%, and even more preferably, at least about 30% identical to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, and preferably to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9. Similarly, an epimerase that has an amino acid sequence that is homologous to an amino acid sequence of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-

galactose 4-epimerase includes any epimerase having an amino acid sequence that is at least about 15%, and preferably at least about 20%, and more preferably at least about 25%, and even more preferably, at least about 30% identical to an amino acid sequence of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase or a UDP-galactose 4-epimerase, and preferably to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10.

According to one embodiment of the present invention, homology or percent identity between two or more nucleic acid or amino acid sequences is performed using methods known in the art for aligning and/or calculating percentage identity. To compare the homology/percent identity between two or more sequences as set forth above, for example, a module contained within DNASTAR (DNASTAR, Inc., Madison, Wisconsin) can be used. In particular, to calculate the percent identity between two nucleic acid or amino acid sequences, the Lipman-Pearson method, provided by the MegAlign module within the DNASTAR program, is preferably used, with the following parameters, also referred to herein as the Lipman-Pearson standard default parameters:

(1) Ktuple = 2;

(2) Gap penalty = 4;

(3) Gap length penalty = 12.

Using the Lipman-Pearson method with these parameters, for example, the percent identity between the amino acid sequence for *E. coli* GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (SEQ ID NO:4) and human GDP-4-keto-6-deoxy-D-mannose epimerase/reductase (FX) (SEQ ID NO:6) is 27.7%, which is comparable to the 27% identity described for these enzymes in Tonetti et al., 1998, *Acta Cryst.* D54:684-686.

According to another embodiment of the present invention, to align two or more nucleic acid or amino acid sequences, for example to generate a consensus sequence or evaluate the similarity at various positions between such sequences, a CLUSTAL alignment program (e.g., CLUSTAL, CLUSTAL V, CLUSTAL W), also available as a module within the DNASTAR program, can be used using the following parameters, also referred to herein as the CLUSTAL standard default parameters:

Multiple Alignment Parameters (i.e., for more than 2 sequences):

(1) Gap penalty = 10;

(2) Gap length penalty = 10;

Pairwise Alignment Parameters (i.e., for two sequences):

(1) Ktuple = 1;

(2) Gap penalty = 3;

(3) Window = 5;

(4) Diagonals saved = 5.

According to the present invention, a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase can be a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from any organism, including *Arabidopsis thaliana*, *Escherichia coli*, and human. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *Arabidopsis thaliana* is represented herein by SEQ ID NO:1. SEQ ID NO:1 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:2. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *Escherichia coli* is represented herein by SEQ ID NO:3. SEQ ID NO:3 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:4. A nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase from *homo sapiens* is represented herein by SEQ ID NO:5. SEQ ID NO:5 encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase having an amino acid sequence represented herein as SEQ ID NO:6.

According to the present invention, a UDP-galactose 4-epimerase can be a UDP-galactose 4-epimerase from any organism, including *Escherichia coli* and human. A nucleic acid sequence encoding a UDP-galactose 4-epimerase from *Escherichia coli* is represented herein by SEQ ID NO:7. SEQ ID NO:7 encodes a UDP-galactose 4-epimerase having an amino acid sequence represented herein as SEQ ID NO:8. A nucleic acid sequence encoding a UDP-galactose 4-epimerase from *homo sapiens* is represented herein by SEQ ID NO:9. SEQ ID NO:9 encodes a UDP-galactose 4-epimerase having an amino acid sequence represented herein as SEQ ID NO:10.

In a preferred embodiment, an epimerase encompassed by the present invention has an amino acid sequence that aligns with the amino acid sequence of SEQ ID NO:11, for example using a CLUSTAL alignment program, wherein amino acid residues in the

amino acid sequence of the epimerase align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11, and preferably at least about 75% of non-Xaa residues in SEQ ID NO:11, and more preferably, at least about 90% of non-Xaa residues in SEQ ID NO:11, and even more preferably 100% of non-Xaa residues in SEQ ID NO:11. The percent identity of residues aligning with 100% identity with non-Xaa residues can be simply calculated by dividing the number of 100% identical matches at non-Xaa residues in SEQ ID NO:11 by the total number of non-Xaa residues in SEQ ID NO:11. A preferred nucleic acid sequence encoding an epimerase encompassed by the present invention include a nucleic acid sequence encoding an epimerase having an amino acid sequence with the above described identity to SEQ ID NO:11. Such an alignment using a CLUSTAL alignment program is based on the same parameters as previously disclosed herein. SEQ ID NO:11 represents a consensus amino acid sequence of an epimerase which was derived by aligning at least portions of amino acid sequences SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8, as described in Somers et al., 1998, *Structure* 6:1601-1612, and can be approximately duplicated using CLUSTAL.

In another embodiment, an epimerase encompassed by the present invention includes an epimerase that has a catalytic site which includes amino acid residues: serine, tyrosine and lysine. Preferably, such serine, tyrosine and lysine residues are located at positions in the epimerase amino acid sequence which align using a CLUSTAL alignment program with positions Ser105, Tyr134 and Lys138 of consensus sequence SEQ ID NO:11, with positions Ser109, Tyr138 and Lys142 of sequence SEQ ID NO:2, with positions Ser107, Tyr136 and Lys140 of SEQ ID NO:4, with positions Ser114, Tyr143 and Lys147 of sequence SEQ ID NO:6, with positions Ser124, Tyr149 and Lys153 of sequence SEQ ID NO:8 or with positions Ser132, Tyr157 and Lys161 of sequence SEQ ID NO:10.

In another embodiment, an epimerase that has an amino acid sequence that is homologous to an amino acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase includes any epimerase that has an amino acid motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly, which is found, for example in positions 8 through 14 of the consensus sequence SEQ ID NO:11, in positions 12 through 18 of SEQ ID NO:2, in positions 10 through 16 of SEQ ID NO:4, in positions 14 through 20 of SEQ ID NO:6, in positions

7 through 13 of SEQ ID NO:8, and in positions 9 through 15 of SEQ ID NO:10. Such a motif can be identified by its alignment with the same motif in the above-identified amino acid sequences using a CLUSTAL alignment program. Preferably, such motif is located within the first 25 N-terminal amino acids of the amino acid sequence of the epimerase.

In yet another embodiment, an epimerase encompassed by the present invention includes an epimerase that has a substrate binding site which includes amino acid residues that align using a CLUSTAL alignment program with at least 50% of amino acid positions Asn177, Ser178, Arg187, Arg209, Lys283, Asn165, Ser107, Ser108, Cys109, Asn133, Tyr136 and His179 of SEQ ID NO:4. Alignment with positions Ser107, Tyr136, Asn165, Arg209, is preferably with 100% identity (i.e., exact match of residue, under parameters for alignment).

In another embodiment of the present invention, an epimerase encompassed by the present invention comprises at least 4 contiguous amino acid residues having 100% identity with at least 4 contiguous amino acid residues of an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:10, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters or by comparing an alignment using a CLUSTAL program with CLUSTAL standard default parameters. According to the present invention, the term "contiguous" means to be connected in an unbroken sequence. For a first sequence to have "100% identity" with a second sequence means that the first sequence exactly matches the second sequence with no gaps between nucleotides or amino acids.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that comprises at least 12 contiguous nucleic acid residues having 100% identity with at least 12 contiguous nucleic acid residues of a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:10, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters or by comparing an alignment using a CLUSTAL program with CLUSTAL standard default parameters.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that hybridizes under stringent

hybridization conditions to a nucleic acid sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9. As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules are used to identify similar nucleic acid molecules. Such standard conditions are disclosed, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989. Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety (see specifically, pages 9.31-9.62). In addition, formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting varying degrees of mismatch of nucleotides are disclosed, for example, in Meinkoth et al., 1984, *Anal. Biochem.* 138, 267-284; Meinkoth et al., *ibid.*, is incorporated by reference herein in its entirety.

More particularly, stringent hybridization and washing conditions, as referred to herein, refer to conditions which permit isolation of nucleic acid molecules having at least about 70% nucleic acid sequence identity with the nucleic acid molecule being used to probe in the hybridization reaction, more particularly at least about 75%, and most particularly at least about 80%. Such conditions will vary, depending on whether DNA:RNA or DNA:DNA hybrids are being formed. Calculated melting temperatures for DNA:DNA hybrids are 10°C less than for DNA:RNA hybrids. In particular embodiments, stringent hybridization conditions for DNA:DNA hybrids include hybridization at an ionic strength of 6X SSC (0.9 M Na^+) at a temperature of between about 20°C and about 35°C, more preferably, between about 28°C and about 40°C, and even more preferably, between about 35°C and about 45°C. In particular embodiments, stringent hybridization conditions for DNA:RNA hybrids include hybridization at an ionic strength of 6X SSC (0.9 M Na^+) at a temperature of between about 30°C and about 45°C, more preferably, between about 38°C and about 50°C, and even more preferably, between about 45°C and about 55°C. These values are based on calculations of a melting temperature for molecules larger than about 100 nucleotides, 0% formamide and a G + C content of about 40%. Alternatively, T_m can be calculated empirically as set forth in Sambrook et al., *supra*, pages 9.31 to 9.62.

In another embodiment of the present invention, an epimerase encompassed by the present invention is encoded by a nucleic acid sequence that comprises a nucleic acid

sequence selected from the group of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:9 or a fragment thereof, wherein the fragment encodes a protein that is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose, such as under physiological conditions. In another embodiment, an epimerase encompassed by the present invention comprises an amino acid sequence selected from the group of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10 or a fragment thereof, wherein the fragment is capable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose. It is to be understood that the nucleic acid sequence encoding the amino acid sequences identified herein can vary due to degeneracies. As used herein, nucleotide degeneracies refers to the phenomenon that one amino acid can be encoded by different nucleotide codons.

One embodiment of the present invention relates to a method to identify an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose. Preferably, such a method is useful for identifying the GDP-D-mannose:GDP-L-galactose epimerase which catalyzes the conversion of GDP-D-mannose to GDP-L-galactose in the endogenous (i.e., naturally occurring L-ascorbic acid biosynthetic pathway of microorganisms and/or plants). Such a method can include the steps of: (a) contacting a source of nucleic acid molecules with an oligonucleotide at least about 12 nucleotides in length under stringent hybridization conditions, wherein the oligonucleotide is identified by its ability to hybridize under stringent hybridization conditions to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5; and, (b) identifying nucleic acid molecules from the source of nucleic acid molecules which hybridize under stringent hybridization conditions to the oligonucleotide. Nucleic acid molecules identified by this method can then be isolated from the source using standard molecular biology techniques. Preferably, the source of nucleic acid molecules is obtained from a microorganism or plant that has an ascorbic acid production pathway. Such a source of nucleic acid molecules can be any source of nucleic acid molecules which can be isolated from an organism and/or which can be screened by hybridization with an oligonucleotide such as a probe or a PCR primer. Such sources include genomic and cDNA libraries and isolated RNA.

In order to screen cDNA libraries from different organisms and to isolate nucleic acid molecules encoding enzymes such as the GDP-D-mannose:GDP-L-galactose epimerase and related epimerases, one can use any of a variety of standard molecular and biochemical techniques. For example, oligonucleotide primers, preferably degenerate primers, can be designed using the most conserved regions of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase nucleic acid sequence, and such primers can be used in a polymerase chain reaction (PCR) protocol to amplify the same or related epimerases, including the GDP-D-mannose:GDP-L-galactose epimerase from the ascorbic acid pathway, from nucleic acids (e.g., genomic or cDNA libraries) isolated from a desired organism (e.g., a microorganism or plant having an L-ascorbic acid pathway). Similarly, oligonucleotide probes can be designed using the most conserved regions of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase nucleic acid sequence and such probe can be used to identify and isolate nucleic acid molecules, such as from a genomic or cDNA library, that hybridize under conditions of low, moderate, or high stringency with the probe.

Alternatively, the GDP-D-mannose:GDP-L-galactose epimerase can be purified from an organism such as *Prototheca*, the N-terminal amino acid sequence can be determined (including the sequence of internal peptide fragments), and this information can be used to design degenerate primers for amplifying a gene fragment from the organism cDNA. This fragment would then be used to probe the cDNA library, and subsequently fragments that hybridize to the probe would be cloned in that organism or another suitable production organism. There is ample precedent for plant enzymes being expressed in an active form in bacteria, such as *E. coli*. Alternatively, yeast are also a suitable candidate for developing a heterologous system for L-ascorbic acid production.

As discussed above in general for increasing the action of an enzyme in the L-ascorbic acid pathway according to the present invention, in one embodiment of the present invention, the action of an epimerase that catalyzes the conversion of GDP-D-mannose to GDP-L-galactose is increased by amplification of the expression (i.e., overexpression) of such an epimerase. Overexpression of an epimerase can be accomplished, for example, by introduction of a recombinant nucleic acid molecule encoding the epimerase. It is preferred that the gene encoding an epimerase according to

the present invention be cloned under control of an artificial promoter. The promoter can be any suitable promoter that will provide a level of epimerase expression required to maintain a sufficient level of L-ascorbic acid in the production organism. Preferred promoters are constitutive (rather than inducible) promoters, since the need for addition of expensive inducers is therefore obviated. The gene dosage (copy number) of a recombinant nucleic acid molecule according to the present invention can be varied according to the requirements for maximum product formation. In one embodiment, the recombinant nucleic acid molecule encoding an epimerase according to the present invention is integrated into the chromosome of the microorganism.

It is another embodiment of the present invention to provide a microorganism having one or more epimerases according to the present invention with improved affinity for its substrate. An epimerase with improved affinity for its substrate can be produced by any suitable method of genetic modification or protein engineering. For example, computer-based protein engineering can be used to design an epimerase protein with greater stability and better affinity for its substrate. See for example, Maulik et al., 1997, *Molecular Biotechnology: Therapeutic Applications and Strategies*, Wiley-Liss, Inc., which is incorporated herein by reference in its entirety.

As noted above, in the method for production of L-ascorbic acid of the present invention, a microorganism having a genetically modified L-ascorbic acid production pathway is cultured in a fermentation medium for production of L-ascorbic acid. An appropriate, or effective, fermentation medium refers to any medium in which a genetically modified microorganism of the present invention, when cultured, is capable of producing L-ascorbic acid. Such a medium is typically an aqueous medium comprising assimilable carbon, nitrogen and phosphate sources. Such a medium can also include appropriate salts, minerals, metals and other nutrients. One advantage of genetically modifying a microorganism as described herein is that although such genetic modifications can significantly alter the production of L-ascorbic acid, they can be designed such that they do not create any nutritional requirements for the production organism. Thus, a minimal-salts medium containing glucose as the sole carbon source can be used as the fermentation medium. The use of a minimal-salts-glucose medium for the L-ascorbic acid fermentation will also facilitate recovery and purification of the L-ascorbic acid product.

In one mode of operation of the present invention, the carbon source concentration, such as the glucose concentration, of the fermentation medium is monitored during fermentation. Glucose concentration of the fermentation medium can be monitored using known techniques, such as, for example, use of the glucose oxidase enzyme test or high pressure liquid chromatography, which can be used to monitor glucose concentration in the supernatant, e.g., a cell-free component of the fermentation medium. As stated previously, the carbon source concentration should be kept below the level at which cell growth inhibition occurs. Although such concentration may vary from organism to organism, for glucose as a carbon source, cell growth inhibition occurs at glucose concentrations greater than at about 60 g/L, and can be determined readily by trial. Accordingly, when glucose is used as a carbon source the glucose concentration in the fermentation medium is maintained in the range of from about 1 g/L to about 100 g/L, more preferably in the range of from about 2 g/L to about 50 g/L, and yet more preferably in the range of from about 5 g/L to about 20 g/L. Although the carbon source concentration can be maintained within desired levels by addition of, for example, a substantially pure glucose solution, it is preferred to maintain the carbon source concentration of the fermentation medium by addition of aliquots of the original fermentation medium. The use of aliquots of the original fermentation medium are desirable because the concentrations of other nutrients in the medium (e.g. the nitrogen and phosphate sources) can be maintained simultaneously. Likewise, the trace metals concentrations can be maintained in the fermentation medium by addition of aliquots of the trace metals solution.

In an embodiment of the fermentation process of the present invention, a fermentation medium is prepared as described above. This fermentation medium is inoculated with

an actively growing culture of genetically modified microorganisms of the present invention in an amount sufficient to produce, after a reasonable growth period, a high cell density. Typical inoculation cell densities are within the range of from about 0.1 g/L to about 15 g/L, preferably from about 0.5 g/L to about 10 g/L and more preferably from about 1 g/L to about 5 g/L, based on the dry weight of the cells. The cells are then grown to a cell density in the range of from about 10 g/L to about 100 g/L preferably from about

20 g/L to about 80 g/L, and more preferably from about 50 g/L to about 70 g/L. The residence times for the microorganisms to reach the desired cell densities during fermentation are typically less than about 200 hours, preferably less than about 120 hours, and more preferably less than about 96 hours.

The microorganisms useful in the method of the present invention can be cultured in conventional fermentation modes, which include, but are not limited to, batch, fed-batch, and continuous. It is preferred, however, that the fermentation be carried out in fed-batch mode. In such a case, during fermentation some of the components of the medium are depleted. It is possible to initiate fermentation with relatively high concentrations of such components so that growth is supported for a period of time before additions are required. The preferred ranges of these components are maintained throughout the fermentation by making additions as levels are depleted by fermentation. Levels of components in the fermentation medium can be monitored by, for example, sampling the fermentation medium periodically and assaying for concentrations. Alternatively, once a standard fermentation procedure is developed, additions can be made at timed intervals corresponding to known levels at particular times throughout the fermentation. As will be recognized by those in the art, the rate of consumption of nutrient increases during fermentation as the cell density of the medium increases. Moreover, to avoid introduction of foreign microorganisms into the fermentation medium, addition is performed using aseptic addition methods, as are known in the art. In addition, a small amount of anti-foaming agent may be added during the fermentation.

The present inventors have determined that high levels of magnesium in the fermentation medium inhibits the production of L-ascorbic acid due to repression of enzymes early in the production pathway, although enzymes late in the pathway (i.e., from L-galactose to L-ascorbic acid) are not negatively affected (See Examples). Therefore, in a preferred embodiment of the method of the present invention, the step of culturing is carried out in a fermentation medium that is magnesium (Mg^{2+}) limited. Even more preferably, the fermentation is magnesium limited during the cell growth phase. Preferably, the fermentation medium comprises less than about 0.5 g/L of Mg^{2+} during the cell growth phase of fermentation, and even more preferably, less than about 0.2 g/L of Mg^{2+}, and even more preferably, less than about 0.1 g/L of Mg^{2+}.

The temperature of the fermentation medium can be any temperature suitable for growth and ascorbic acid production, and may be modified according to the growth requirements of the production microorganism used. For example, prior to inoculation of the fermentation medium with an inoculum, the fermentation medium can be brought to and maintained at a temperature in the range of from about 20°C to about 45°C, preferably to a temperature in the range of from about 25°C to about 40°C, and more preferably in the range of from about 30°C to about 38°C.

It is a further embodiment of the present invention to supplement and/or control other components and parameters of the fermentation medium, as necessary to maintain and/or enhance the production of L-ascorbic acid by a production organism. For example, in one embodiment, the pH of the fermentation medium is monitored for fluctuations in pH. In the fermentation method of the present invention, the pH is preferably maintained at a pH of from about pH 6.0 to about pH 8.0, and more preferably, at about pH 7.0. In the method of the present invention, if the starting pH of the fermentation medium is pH 7.0, the pH of the fermentation medium is monitored for significant variations from pH 7.0, and is adjusted accordingly, for example, by the addition of sodium hydroxide. In a preferred embodiment of the present invention, genetically modified microorganisms useful for production of L-ascorbic acid include acid-tolerant microorganisms. Such microorganisms include, for example, microalgae of the genera *Prototheca* and *Chlorella* (See U.S. Patent No. 5,792,631, *ibid.* and U.S. Patent No. 5,900,370, *ibid.*).

The production of ascorbic acid by culturing acid-tolerant microorganisms provides significant advantages over known ascorbic acid production methods. One such advantage is that such organisms are acidophilic, allowing fermentation to be carried out under low pH conditions, with the fermentation medium pH typically less than about 6. Below this pH, extracellular ascorbic acid produced by the microorganism during fermentation is relatively stable because the rate of oxidation of ascorbic acid in the fermentation medium by oxygen is reduced. Accordingly, high productivity levels can be obtained for producing L-ascorbic acid with acid-tolerant microorganisms according to the methods of the present invention. In addition, control of the dissolved oxygen content to very low levels to avoid oxidation of ascorbic acid is unnecessary. Moreover, this

advantage allows for the use of continuous recovery methods because extracellular medium can be treated to recover the ascorbic acid product.

Thus, the present method can be conducted at low pH when acid-tolerant microorganisms are used as production organisms. The benefit of this process is that at low pH, extracellular ascorbic acid produced by the organism is degraded at a reduced rate than if the fermentation medium was at higher pH. For example, prior to inoculation of the fermentation medium with an inoculum, the pH of the fermentation medium can be adjusted, and further monitored during fermentation. Typically, the pH of the fermentation medium is brought to and maintained below about 6, preferably below 5.5, and more preferably below about 5. The pH of the fermentation medium can be controlled by the addition of ammonia to the fermentation medium. In such cases when ammonia is used to control pH, it also conveniently serves as a nitrogen source in the fermentation medium.

The fermentation medium can also be maintained to have a dissolved oxygen content during the course of fermentation to maintain cell growth and to maintain cell metabolism for L-ascorbic acid formation. The oxygen concentration of the fermentation medium can be monitored using known methods, such as through the use of an oxygen probe electrode. Oxygen can be added to the fermentation medium using methods known in the art, for example, through agitation and aeration of the medium by stirring or shaking. Preferably, the oxygen concentration in the fermentation medium is in the range of from about 20% to about 100% of the saturation value of oxygen in the medium based upon the solubility of oxygen in the fermentation medium at atmospheric pressure and at a temperature in the range of from about 30°C to about 40°C. Periodic drops in the oxygen concentration below this range may occur during fermentation, however, without adversely affecting the fermentation.

The genetically modified microorganisms of the present invention are engineered to produce significant quantities of extracellular L-ascorbic acid. Extracellular L-ascorbic acid can be recovered from the fermentation medium using conventional separation and purification techniques. For example, the fermentation medium can be filtered or centrifuged to remove microorganisms, cell debris and other particulate matter, and L-ascorbic acid can be recovered from the cell-free supernate by conventional methods, such

as, for example, ion exchange, chromatography, extraction, solvent extraction, membrane separation, electrodialysis, reverse osmosis, distillation, chemical derivatization and crystallization.

One such example of L-ascorbic acid recovery is provided in U.S. Patent No. 4,595,659 by Cayle, incorporated herein in its entirety be reference, which discloses the isolation of L-ascorbic acid from an aqueous fermentation medium by ion exchange resin adsorption and elution, which is followed by decoloration, evaporation and crystallization. Further, isolation of the structurally similar isoascorbic acid from fermentation medium by a continuous multi-bed extraction system of anion-exchange resins is described by K. Shimizu, *Agr. Biol. Chem.* 31:346-353 (1967), which is incorporated herein in its entirety by reference.

Intracellular L-ascorbic acid produced in accordance with the present invention can also be recovered and used in a variety of applications. For example, cells from the microorganisms can be lysed and the ascorbic acid which is released can be recovered by a variety of known techniques. Alternatively, intracellular ascorbic acid can be recovered by washing the cells to extract the ascorbic acid, such as through diafiltration.

Development of a microorganism with enhanced ability to produce L-ascorbic acid by genetic modification can be accomplished using both classical strain development and molecular genetic techniques, and particularly, recombinant technology (genetic engineering). In general, the strategy for creating a microorganism with enhanced L-ascorbic acid production is to (1) inactivate or delete at least one, and preferably more than one of the competing or inhibitory pathways in which production of L-ascorbic acid is negatively affected (e.g., inhibited), and more significantly to (2) amplify the L-ascorbic acid production pathway by increasing the action of a gene(s) encoding an enzyme(s) involved in the pathway.

In one embodiment, the strategy for creating a microorganism with enhanced L-ascorbic acid production is to amplify the L-ascorbic acid production pathway by increasing the action of GDP-D-mannose:GDP-L-galactose epimerase, as discussed above. Such strategy includes genetically modifying the endogenous GDP-D-mannose:GDP-L-galactose epimerase such that L-ascorbic acid production is increased, and/or expressing/overexpressing a recombinant epimerase that catalyzes the conversion

of GDP-D-mannose to GDP-L-galactose, which includes expression of recombinant GDP-D-mannose:GDP-L-galactose epimerase and/or homologues thereof, and of other recombinant epimerases such as GDP-4-keto-6-deoxy-D-mannose epimerase reductase and epimerases that share structural homology with such epimerase as discussed in detail above.

It is to be understood that a production organism can be genetically modified by recombinant technology in which a nucleic acid molecule encoding a protein involved in the L-ascorbic acid production pathway disclosed herein is transformed into a suitable host which is a different member of the plant kingdom from which the nucleic acid molecule was derived. For example, it is an embodiment of the present invention that a recombinant nucleic acid molecule encoding a GDP-D-mannose:GDP-L-galactose epimerase from a higher plant can be transformed into a microalgal host in order to overexpress the epimerase and enhance production of L-ascorbic acid in the microalgal production organism.

As previously discussed herein, in one embodiment, a genetically modified microorganism can be a microorganism in which nucleic acid molecules have been deleted, inserted or modified, such as by insertion, deletion, substitution, and/or inversion of nucleotides, in such a manner that such modifications provide the desired effect within the microorganism. A genetically modified microorganism is preferably modified by recombinant technology, such as by introduction of an isolated nucleic acid molecule into a microorganism. For example, a genetically modified microorganism can be transfected with a recombinant nucleic acid molecule encoding a protein of interest, such as a protein for which increased expression is desired. The transfected nucleic acid molecule can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transfected (i.e., recombinant) host cell in such a manner that its ability to be expressed is retained. Preferably, once a host cell of the present invention is transfected with a nucleic acid molecule, the nucleic acid molecule is integrated into the host cell genome. A significant advantage of integration is that the nucleic acid molecule is stably maintained in the cell. In a preferred embodiment, the integrated nucleic acid molecule is operatively linked to a transcription control sequence (described below) which can be induced to control expression of the nucleic acid molecule.

A nucleic acid molecule can be integrated into the genome of the host cell either by random or targeted integration. Such methods of integration are known in the art. For example, an *E. coli* strain ATCC 47002 contains mutations that confer upon it an inability to maintain plasmids which contain a ColE1 origin of replication. When such plasmids are transferred to this strain, selection for genetic markers contained on the plasmid results in integration of the plasmid into the chromosome. This strain can be transformed, for example, with plasmids containing the gene of interest and a selectable marker flanked by the 5'- and 3'-termini of the *E. coli lacZ* gene. The *lacZ* sequences target the incoming DNA to the *lacZ* gene contained in the chromosome. Integration at the *lacZ* locus replaces the intact *lacZ* gene, which encodes the enzyme β-galactosidase, with a partial *lacZ* gene interrupted by the gene of interest. Successful integrants can be selected for β-galactosidase negativity.

A genetically modified microorganism can also be produced by introducing nucleic acid molecules into a recipient cell genome by a method such as by using a transducing bacteriophage. The use of recombinant technology and transducing bacteriophage technology to produce several different genetically modified microorganism of the present invention is known in the art.

According to the present invention, a gene, for example the GDP-D-mannose:GDP-L-galactose epimerase gene, includes all nucleic acid sequences related to a natural epimerase gene such as regulatory regions that control production of the epimerase protein encoded by that gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself. In another embodiment, a gene, for example the GDP-D-mannose:GDP-L-galactose epimerase gene, can be an allelic variant that includes a similar but not identical sequence to the nucleic acid sequence encoding a given GDP-D-mannose:GDP-L-galactose epimerase gene. An allelic variant of a GDP-D-mannose:GDP-L-galactose epimerase gene which has a given nucleic acid sequence is a gene that occurs at essentially the same locus (or loci) in the genome as the gene having the given nucleic acid sequence, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Allelic variants typically encode proteins having similar activity to that of the protein encoded by the gene to which they are being

compared. Allelic variants can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions). Allelic variants are well known to those skilled in the art and would be expected to be found within a given microorganism or plant and/or among a group of two or more microorganisms or plants.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to human manipulation). As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA. There is no limit, other than a practical limit, on the maximal size of a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes, or portions thereof.

An isolated nucleic acid molecule of the present invention can be obtained from its natural source either as an entire (i.e., complete) gene or a portion thereof capable of forming a stable hybrid with that gene. An isolated nucleic acid molecule can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning) or chemical synthesis. Isolated nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications provide the desired effect within the microorganism. A structural homologue of a nucleic acid sequence has been described in detail above. Preferably, a homologue of a nucleic acid sequence encodes a protein which has an amino acid sequence that is sufficiently similar to the natural protein amino acid sequence that a nucleic acid sequence encoding the homologue is capable of hybridizing under stringent conditions to (i.e., with) a nucleic acid molecule encoding the natural protein (i.e., to the complement of the nucleic acid strand encoding the natural protein amino acid sequence). A nucleic acid molecule homologue encodes a protein homologue. As used herein, a homologue protein includes proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation,

amidation and/or addition of glycosylphosphatidyl inositol) in such a manner that such modifications provide the desired effect on the protein and/or within the microorganism (e.g., increased or decreased action of the protein).

A nucleic acid molecule homologue can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *ibid.*). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, PCR amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecule homologues can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid and/or by hybridization with a wild-type gene.

Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a gene involved in an L-ascorbic acid production pathway.

Knowing the nucleic acid sequences of certain nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules and/or (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions). Such nucleic acid molecules can be obtained in a variety of ways including traditional cloning techniques using oligonucleotide probes to screen appropriate libraries or DNA and PCR amplification of appropriate libraries or DNA using oligonucleotide primers. Preferred libraries to screen or from which to amplify nucleic acid molecule include bacterial and yeast genomic DNA libraries, and in particular, microalgal genomic DNA libraries. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid.*

The present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host microorganism of the present invention. Such a vector can contain nucleic acid sequences that are not naturally found adjacent to the isolated nucleic acid molecules to be inserted into the vector. The vector can be either RNA or DNA and typically is a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of nucleic acid molecules. One type of recombinant vector, referred to herein as a recombinant molecule and described in more detail below, can be used in the expression of nucleic acid molecules. Preferred recombinant vectors are capable of replicating in a transformed bacterial cells, yeast cells, and in particular, in microalgal cells.

Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection and biolistics.

A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules operatively linked to an expression vector containing one or more transcription control sequences. The phrase, operatively linked, refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. In the present invention, expression vectors are typically plasmids. Expression vectors of the present invention include any vectors that function (i.e., direct gene expression) in a yeast host cell, a bacterial host cell, and preferably a microalgal host cell.

Nucleic acid molecules of the present invention can be operatively linked to expression vectors containing regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression

of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in yeast or bacterial cells or preferably, in microalgal cells. A variety of such transcription control sequences are known to those skilled in the art.

It may be appreciated by one skilled in the art that use of recombinant DNA technologies can improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into the host cell chromosome, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals, modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

The following experimental results are provided for the purposes of illustration and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

The present example describes the elucidation of the pathway from glucose to L-ascorbic acid through GDP-D-mannose in plants.

Since the present inventors have previously shown that *Prototheca* makes L-ascorbic acid (AA) from glucose, it was worthwhile to examine cultures for some of the early conversion products of glucose. In the past, the present inventors had concentrated on pathways from glucose to organic acids, based on the published pathway of L-ascorbic acid synthesis in animals and proposed pathways in plants. The present inventors demonstrate herein that the pathway from glucose to L-ascorbic acid involves not organic acids, but rather sugar phosphates and nucleotide diphosphate sugars (NDP-sugars).

Prior to the present invention, it was known that all cells synthesize polysaccharides by first forming NDP-sugars. The sugar moiety is then incorporated into polymer, while the cleaved NDP is recycled. A variety of polysaccharides are known, and are usually named based on the relative proportions of the sugar residues in the polymers. For example, a "galactomannan" contains mostly galactose, and to a lesser degree, mannose residues. The "biopolymer" from *Prototheca* strains isolated by the present inventors was analyzed and found to be 80% D-galactose, 18% rhamnose (D- or L-configuration not determined), and 2% L-arabinose. The present inventors provide evidence herein of how the respective NDP-sugars that make up the *Prototheca* biopolymer are formed, and what correlations exist between L-ascorbic acid synthesis and the formation of the NDP-sugar forms of the sugar residues found in the biopolymer.

The common NDP-sugar UDP-glucose is shown in Fig. 2B. This is formed in plants from glucose-1-P by the action of UDP-D-glucose pyrophosphorylase. UDP-glucose can be epimerized in plants to form UDP-D-galactose, using UDP-D-glucose-4-epimerase. UDP-D-galactose can also be formed by phosphorylation of D-galactose by galactokinase to form D-galactose-1-P, which can be converted to UDP-D-galactose by UDP-D-galactose pyrophosphorylase. These known routes were believed to account for the D-galactose in the *Prototheca* biopolymer. The UDP-L-arabinose can be formed by known reactions beginning with the oxidation of UDP-D-glucose to UDP-D-glucuronic acid (by UDP-D-glucose dehydrogenase), decarboxylation to UDP-D-xylose, and epimerization to UDP-L-arabinose. This accounts for the arabinose residues in the

biopolymer. UDP-L-rhamnose is known to be formed from UDP-D-glucose, thus all three of the sugar moieties in the *Prototheca* biopolymer can be accounted for by a pathway through glucose-1-P and UDP-glucose. Alternatively, if the rhamnose in the biopolymer is D-rhamnose, it is not formed via UDP-D-glucose, but by oxidation of GDP-D-mannose (See Fig. 1).

GDP-D-rhamnose is formed by converting glucose, in turn, to D-glucose-6-P, D-fructose-6-P, D-mannose-6-P, D-mannose-1-P, GDP-D-mannose, and GDP-D-rhamnose. It was of interest to the present inventors that this route passes through GDP-D-mannose. Exogenous mannose is known to be converted to D-mannose-6-P in plants, and can enter the path above. D-mannose is converted to L-ascorbic acid by *Prototheca* cells cultured by the present inventors as well or better than glucose (see Example 4). The mechanism of conversion, in *Chlorella pyrenoidosa* , of GDP-D-mannose to GDP-L-galactose by GDP-D-mannose:GDP-L-galactose epimerase, has been known for years (See, Barber, 1971, *Arch. Biochem. Biophys.* 147:619-623, incorporated herein by reference in its entirety). The present inventors have discovered herein that L-galactose and L-galactono-γ-lactone are rapidly converted to L-ascorbic acid by strains *of Prototheca* and *Chlorella pyrenoidosa*. Prior to the present invention, it was known that L-galactono-γ-lactone is converted to L-ascorbic acid in several plant systems, but the synthesis steps prior to this step were unknown. Based on the published literature and the present experimental evidence, the present inventors have determined that the L-ascorbic acid biosynthetic pathway in plants passes through GDP-D-mannose and involves sugar phosphates and NDP-sugars. The proposed pathway is shown in Fig. 1. Salient points relevant to the design and production of genetically modified microorganisms useful in the present method include:

1. The enzymes leading from D-glucose to D-fructose-6-P are well known enzymes in the first, uncommitted steps of glycolysis.

2. The enzymes involved in the conversion of D-fructose-6-P to GDP-D-mannose have been well characterized in plants, yeast, and bacteria, particularly *Azotobacter vinelandii and Pseudomonas aeruginosa,* which convert GDP-D-mannose to GDP-D-mannuronic acid, which is the precursor for alginate (See for example, Sa-Correia et al., 1987, *J. Bacteriol.* 169:3224-3231; Koplin et al., 1992, *J. Bacteriol.* 174:191-199; Oesterhelt et al., 1996, *Plant Science* 121:19-27; Feingold et al., 1980, The

Biochemistry of Plants: Vol 3: Carbohydrates, structure and function, P.K. Stampf & E.E. Conn, eds., Academic Press, New York, pp. 101-170; Smith et al., 1992, *Mol. Cell Biol.* 12:2924-2930; Boles et al., 1994, *Eur. J. Med.* 220:83-96; Hashimoto et al., 1997, *J. Biol. Chem.* 272:16308-16314, all of which are incorporated herein by reference in their entirety).

3. Barber (1971, *supra*, and 1975) identified in *Chlorella pyrenoidosa* the enzyme activities for the conversion of GDP-D-mannose to GDP-L-galactose and L-galactose-l-P.

4. The present inventors have shown herein the rapid conversion of L-galactose and L-galactono-γ-lactone to L-ascorbic acid by *Prototheca* cells.

5. L-galactono-γ-lactone and L-galactonic acid can be interconverted in solution by changing the pH of the solution; addition of base shifts the equilibrium to L-galactonic acid, while addition of acid shifts the equilibrium to the lactone. Cells may have an enzymatic means for this conversion in addition to this non-enzymatic route.

6. In plants, GDP-L-fucose is also formed from GDP-D-mannose, presumably for incorporation into polysaccharide. Roberts (1971) fed labeled D-mannose to corn root tips and found the label in polysaccharide, specifically in the residues of D-mannose, L-galactose, and L-fucose. No label was detected in D-glucose, D-galactose, L-arabinose, or D-xylose. *Prototheca and C. pyrenoidosa* cells have the ability to convert L-fucose (6-deoxy-L-galactose) to a dipyridyl-positive product that was shown by HPLC not to be L-ascorbic acid. The present inventors believe that it is was the 6-deoxy analog of L-ascorbic acid.

Example 2

This example shows that in *Prototheca*, like other plants (Loewus, F.A. 1988. In: J. Priess (ed.), The Biochemistry of Plants, 14:85-107. New York, Academic Press) and the green microalga *Chlorella pyrenoidosa* (Renstrom, *et al.*, 1983. Plant Sci. Lett. 28:299-305), ascorbic acid (AA) production from glucose proceeds by a biosynthetic pathway that allows retention of the configuration of the carbon skeleton of glucose.

Cultures of the strain UV77-247 were grown to moderate cell density in shake flasks with 1-^{13}C-labeled glucose as 10% of the total glucose (40 g/L). Incubation was

as per the standard Mg-limited screen (see Example 3). The culture supernates were clarified, deionized to remove salts, lyophilized, and subjected to nuclear magnetic resonance (nmr) analysis to determine where in the AA molecule the ^{13}C was located. In each case, approximately 85% of the label was found at the C-1 position of AA, with most of the remaining label at the C-6 position. This strongly indicated that AA is synthesized from glucose by a pathway that retains the carbon chain configuration, *i. e.*, C-1 of glucose becomes C-1 of AA. This has typically been observed in plants (Loewus, F.A. 1988. Ascorbic acid and its metabolic products. In: The Biochemistry of Plants, ed. J. Priess, 14:85-107. New York, Academic Press). Animals (Mapson, L.W. and F.A. Isherwood 1956. Biochem. J. 64:151-157; Loewus, F.A. 1960. J. Biol. Chem. 235(4):937-939) and protists such as *Euglena* (Shigeoka, S., *et al.*, 1979. J. Nutr. Sci. Vitaminol. 25:299-307), on the other hand, synthesize AA by a pathway that involves the inversion of configuration, *i. e.*, C-1 of glucose becomes C-6 of AA. Demonstration of the inversion/non-inversion nature of the pathway was an important step in determining the pathway of AA biosynthesis since the two types of pathways require different types of enzymatic reactions. The label found at C-6 of AA is thought to be due to metabolism of glucose and subsequent gluconeogenesis. The metabolism of glucose in glycolysis proceeds through triose-phosphate intermediates. After this, the C-1 and C-6 carbons of glucose become biochemically equivalent. Hexose phosphates can be regenerated from the triose phosphates by gluconeogenesis, which is essentially a reversal of the degradative pathway. Consequently, metabolism of C-1-labeled glucose to triose phosphates with subsequent gluconeogenesis would result in the formation of hexose phosphate molecules labeled at either or both C-1 and C-6. If those hexose phosphates were precursors to AA, one would expect the AA to be similarly labeled. Consistent with this type of "isotopic mixing" is the observation that sucrose obtained from 1-^{13}C-labeled glucose was labeled at positions 1, 6, 1' and 6'.

Glucose can also be metabolized by the pentose phosphate pathway, the overall balanced equation for which is:

3 Glucose-6-phosphate → 2 Fructose-6-phosphate + Glyceraldehyde-3-phosphate + 3 CO_2

Based on the known biochemistry, it would then be expected that the label at each of the carbons in glucose (Table 1 left column) would appear at the positions for the other molecules shown, and that these patterns would be reflected in the AA formed from C-2- and C-3-labeled glucose.

TABLE 1

Predicted Carbon Labeling of Metabolites of Glucose in the Pentose Phosphate Pathway

Labeled Glucose Carbon	Position of Labeled Carbon in:			
	CO_2	F6P(1)	F6P(2)	G3P
1	+	-	-	-
2	-	1,3	1	-
3	-	2	2,3	-
4	-	4	4	1
5	-	5	5	2
6	-	6	6	3

AA recovered from cultures fed glucose labeled at C-2 or C-3 was also analyzed for its labeling patterns (Table 2).

TABLE 2

Labeling Pattern in AA after Cells were Fed 2-^{13}C and 3-^{13}C-glucose

Carbon Position in AA	Isotopic enhancement after growth on:	
	C-2 labeled glucose	C-3 labeled glucose
1	1.0	0.4
2	10.0	0.9
3	0.5	9.9
4	0	2.8
5	2.2	0.2
6	0	0

The data above again suggest a pathway from glucose to AA that proceeds by retention of configuration. As in the experiments with C-1 labeled glucose, approximately one-fifth of the label is present in "mirror image" position to the glucose label (C-5 for C-2 labeled glucose and C-4 for C-3 labeled glucose), indicating levels of gluconeogenesis consistent with those previously observed.

The small, but significant amount of enhancement observed in other positions is consistent with flux through the pentose phosphate pathway. As predicted above, carbon

flux through this pathway would result in isotopic enhancement at positions 1 and 3 when cells were grown on 2-^{13}C glucose and enhancement at position 2 when cells were grown on 3-^{13}C glucose. This is indeed observed. That there is twice as much enhancement at C-1 as there is at C-3 after growth on 2-^{13}C glucose is also predicted. These data indicate a small but measurable amount of carbon flux through the pentose phosphate pathway.

Example 3

This example shows the methods for generating, screening and isolating mutants of *Prototheca* with altered AA productivities compared to the starting strain ATCC 75669.

ATCC No. 75669, identified as *Prototheca moriformis* RSP1385 (unicellular green microalga), was deposited on February 8, 1994, with the American Type Culture Collection (ATCC), Rockville, Maryland, 20852, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. Initial screening of *Prototheca* species and strains was reported in U.S. Patent No. 5,900,370, *ibid.* Table 3 lists the formulations of the media for growth and maintenance of the strains. Glucose for fermentors was supplied as glucose monohydrate and calculated on an anhydrous basis. The recipe for the trace metals solution is given in Table 4. The standard growth temperature was 35°C. All organisms were cultured axenically.

TABLE 3
Media for Growth and Maintenance of *Prototheca* Strains
All quantities are in g/L unless otherwise specified

Ingredient	Liquid		Agar		
	Standard	Mg-limiting	Slants	Ferrozine Plates	Standard Plates
Potassium phosphate monobasic	1.3	1.3	2.0	0.27	2.0
Potassium phosphate dibasic	3.8	3.8	2.0	1.4	2.0
Trisodium citrate dihydrate	7.7	7.7	2.6	1.3	2.6
Magnesium sulfate heptahydrate	0.40	0.02	0.4	0.01	0.4
Ammonium sulfate	3.7	3.7	1.0	1.0	1.0
Trace Metals Solution	2 mL	2 mL	2 mL	2 mL	2 mL
Ferrous sulfate heptahydrate	1.5 mg	4.5 mg	1.5 mg	-	1.5 mg
Calcium chloride dihydrate	-	0.25	-	-	-

Ingredient	Liquid		Agar		
	Standard	Mg-limiting	Slants	Ferrozine Plates	Standard Plates
Manganous sulfate monohydrate	-	0.08	-	-	-
Yeast extract	-	-	2.5	-	-
Agar	-	-	15	15 (Noble)	15
pH before autoclaving	7.2	7.2	7.2	7.2	7.2
Autoclave, then add					
Copper sulfate, pentahydrate, 100 g/L	-	-	-	2 mL	-
40 g/L Ferrozine in 5 mM phosphate (pH 7.5 final)	-	-	-	8.8 mL	-
Ferric ammonium sulfate dodecahydrate, 40 g/L	-	-	-	3.8 mL	-
50% glucose with 25 mg/L thiamine HCl	40 mL	60 mL	10 mL	10 mL	10 mL

TABLE 4

Trace Metals Solution

Compound	Molecular Weight	Conc. of Individ. Solutions, g/L	mL Indiv. Stock per liter of Working Stock
Distilled Water	—	—	823
Hydrochloric Acid	—	Conc.	20
Cobalt Chloride hexahydrate	237.9	24.0	6.5
Boric acid	61.8	38.1	24
Zinc sulfate heptahydrate	287.5	35.3	50
Manganous sulfate monohydrate	169.0	24.6	50
Sodium molybdate dihydrate	242.0	23.8	2.0
Calcium chloride dihydrate	147.0	—	11.4 g
Vanadyl sulfate dihydrate	199.0	10.0	8.0
Nickel nitrate hexahydrate	290.8	5.0	8.0
Sodium selenite	173.0	5.0	8.0

Mutant isolates were generated by treatment with one or more of the following agents: nitrous acid (NA); ethyl methane sulfonate (EMS); or ultraviolet light (UV). Typically, glucose-depleted cells grown in standard liquid medium were washed and resuspended in 25 mM phosphate buffer, pH 7.2, diluted to approximately 10^7 colony-forming units per mL (cfu/mL), exposed to the mutagen to achieve about 99% kill, incubated 4-8 hours in the dark, and spread onto standard agar medium, or agar media containing differential agents.

Some mutant colonies on standard agar medium were picked randomly and subcultured to master plates. Other isolation plates were inverted over chloroform to lyse cells on the surface of the colonies and allow them to release AA. Released AA was detected by spraying the treated plates with a solution of 2,6-dichrorophenol-indophenol (1.25 g/L in 70% EtOH). The ability of AA to reduce this blue redox dye to its colorless form is the basis for a standard assay of AA (Omaye, *et al.*, 1979. Meth. Enzymol. 62:3-11.). Colonies derived from mutagenized cells were saved to master plates for further evaluation if their clear halos were significantly larger than the halos typical of the other mutants in that group. Other mutagenized cells were spread onto plates containing an AA detection system incorporated directly into the agar. This system is based on the ability of AA to reduce ferric iron to ferrous iron. The compound ferrozine (3-(2-pyridyl)-5,6- bis(4-phenylsulfonic acid)-1,2,4-triazine) was present in the agar to complex with the ferrous iron and give a violet color reaction. The ferrozine agar formulation is shown in Table 3. Colonies giving the darkest color reactions were master-plated. When screening for non-AA-producing strains (blocked mutants), white colonies were chosen against a background of relatively dark colonies.

For primary screening of tube cultures, cells were inoculated from master plates into 4 mL of Mg-limiting medium in 16 x 125 mm test tubes, and tubes were shaken in a slanted position on a rotary shaker at 300 rpm for four days. After both three and four days of incubation aliquots were removed for AA assay and cell density determination. Those for AA assay were centrifuged at 1500 x g for 5 min and the resulting supernates were removed for either colorimetric assay or high pressure liquid chromatography (HPLC). Promising isolates were retested in tube culture. Those passing the tube screen were tested in shake flasks.

For secondary screening of flask cultures, cells were inoculated into 50 mL of standard flask medium in 250 mL baffled shake flasks, and incubated on a rotary shaker at 180 rpm until glucose depletion (24-48 hours). A second series of flasks of Mg-sufficient standard medium was inoculated from the first set to a cell density of 0.15 A_{620}, and incubated for 24 hours. A third series of Mg-limiting flask medium was inoculated from the second set by a 1/50 dilution and incubated for 96 hours. Flasks were sampled for AA analysis and cell density measurements during this time as required.

Aliquots for supernatant AA analysis were centrifuged at 5000 x g for 5 min. Aliquots for total whole broth AA analysis were first extracted for 15 min with an equal volume of 5% trichloroacetic acid (TCA) before centrifugation. Aliquots of the resulting supernates were removed for either colorimetric assay or HPLC analysis.

For colorimetric assay of AA, a modification of the method of Omaye, *et al.* (1979. Meth. Enzymol. 62:3-11) was used. Twenty-five µL aliquots of culture supernates were added to wells of 96-well microplates, and 125 µL of color reagent was added. The color reagent consisted of four parts 0.5% aqueous 2,2'-dipyridyl and one part 8.3 mM ferric ammonium sulfate in 27 % (v/v) *o*-phosphoric acid, the two components being mixed immediately before use. After one hour, the absorbance at 520 nm was read. AA concentration was calculated by comparison of the absorbances of AA standards.

HPLC analysis was based on that of Running, *et al.*, (1994). Supernates were chromatographed on a Bio-Rad HPX-87H organic acid column (Bio-Rad Laboratories, Richmond, CA) with 13 mM nitric acid as solvent, at a flow rate of 0.7 mL/min at room temperature. Detection was at either 254 nm using a Waters 441 detector (Millipore Corp., Milford, MA), or at 245 nm using a Waters 481 detector. This system can distinguish between the L- and D- isomers of AA.

For dry weight determinations of cell density, 5 mL whole broth samples were centrifuged at 5000 x g for 5 min, washed once with distilled water, and the pellet was washed into a tared aluminum weighing pan. Cells were dried for 8-24 h at 105°C. Cell weight was calculated by difference.

Table 5 shows the abilities of various mutants of *Prototheca* to synthesize AA.

TABLE 5

AA Synthesizing Ability of Various *Prototheca* Mutants in Flask Screen

Strain	Specific AA Formation, mg AA per L/Culture A_{620}, during Mg-limited Incubation	
	2 Days Incubation	4 Days Incubation
ATCC 75669	22	35
EMS13-4	79	166
UV213-1	0	0
UV218-1	0	0
UV244-1	0	0

Strain	Specific AA Formation, mg AA per L/Culture A_{620}, during Mg-limited Incubation	
	2 Days Incubation	4 Days Incubation
UV244-15	58	68
UV77-247	56	83
UV140-1	67	100
UV164-6	91	131
NA21-14	27	78
UV82-21	0	0
UV127-10	50	95
SP2-3	3	4

The genealogy of these isolates is presented graphically in the "family tree" of Fig. 3. ATCC No. _____, identified as *Prototheca moriformis* EMS13-4 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. ATCC No. _____, identified as *Prototheca moriformis* UV127-10 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. ATCC No. _____, identified as *Prototheca moriformis* SP2-3 (unicellular green microalga), was deposited on May 25, 1999, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure.

Example 4

The following example shows that both growing and resting cells of *Prototheca* can rapidly convert L-galactose and L-galactono-γ-lactone to AA, and that conversion of D-mannose to AA by *Prototheca* is more rapid than conversion of D-glucose.

Shake flask cultures of the mutant strain UV77-247 were grown to glucose depletion in standard liquid medium (Table 3). Cells were washed twice and resuspended in complete medium with the glucose substituted by one of the compounds listed below.

Cell suspensions were incubated for 24 hours at 35° C with shaking, and the entire suspension was extracted with TCA as above and assayed for AA.

Tables 6-8 show that both growing and resting cells of strain UV77-247 can rapidly convert L-galactose and L-galactono-γ-lactone to AA. In these experiments, D-fructose and D-galactose were converted to AA at the same rate as D-glucose, suggesting that they are metabolized to AA through the same route as D-glucose. None of the organic acids suggested in the literature to be intermediates in the biosynthesis of AA were converted to AA, including sorbosone, which has been proposed as an intermediate by Saito *et al.*(1990 Plant Physiol. 94:1496-1500).

TABLE 6

Conversion of Compounds by Resting Cells of Strain UV77-247

Substrate (50 mM)	Total AA, mg/L	AA Relative to No Substrate Control
L-galactose	965	623
L-galactono-γ-lactone	818	476
D-fructose	590	248
D-glucosone	589	247
D-glucose	584	242
D-galactose	542	200
D-glucose (10 mM)	388	46
D-gluconolactone	382	40
D-gulono-γ-lactone	366	24
D-glucuronate	364	22
L-sorbosone	342	0
None	342	0
2-keto-D-gluconic acid	341	-1
D-isoascorbic acid (10 mM)	330	-12
D-glucuronolactone	329	-13
D-gluconic acid	309	-33
D-galacturonic acid	297	-45
L-idonate	296	-46

Since strain UV77-247 converted L-galactose and L-galactono-γ-lactone to AA much more rapidly than it did glucose, it suggests that these compounds are intermediates in the AA biosynthetic pathway and that they are "downstream" from glucose.

The data in Tables 7 and 8 also show that growing and resting cells of UV77-247 consistently convert D-mannose to AA at a rate greater than that of glucose.

TABLE 7

Conversion of Compounds to AA by Resting Cells of Strain UV77-247

Compound	Ascorbic Acid, mg/L		
	25.5 h	30 h	47 h
L-galactose	667	718	620
L-galactono-γ-lactone	644	681	749
D-glucosone	465	462	354
D-mannose	448	462	399
D-fructose	402	408	367
d-glucose	395	404	351
D-galactose	352	361	337
none	287	288	258

TABLE 8

Conversion of Compounds to AA by Growing Cells of Strain UV77-247

Compound	Ascorbic Acid, mg/L		A_{620}	AA/A_{620}
	25.5 h	44 h		
L-galactose	249	506	4.5	112
D-mannose	228	488	5.6	87
L-galactono-γ-lactone	214	342	5.0	68
D-glucose	178	398	5.9	67
D-fructose	181	383	5.9	65
D-glucosone	176	362	5.7	64
D-galactose	185	380	5.9	64
none	182	249	4.4	57
D-gluconic acid (K)	178	262	5.0	52
L-idonate (Na)	182	232	4.7	49
2-keto-D-gluconic acid	182	255	5.3	48
2-deoxy-D-glucose	181	227	4.8	47
D-glucuronic acid lactone	165	218	5.0	44
D-glucuronic acid (Na)	173	241	5.6	43
L-gulono-γ-lactone	152	195	5.0	39
L-sorbosone	178	160	4.7	34
D-glucono-δ-lactone	130	190	5.7	33
D-galacturonic acid	130	180	6.0	30

These cells converted L-galactose, L-galactono-γ-lactone and D-mannose to AA more rapidly than they did glucose, suggesting that mannose exerts its effect in the biosynthetic pathway "downstream" from glucose.

Example 5

Using the methods described above, a collection of mutants was assembled. The specific AA formation for representative mutants are shown in Table 5. The genealogy of these isolates is presented graphically in the "family tree" of Fig. 3.

These isolates were tested for their ability to convert compounds which could be converted to AA by strain UV77-247. Testing was done as in Example 4. Results are shown in Table 9.

TABLE 9

Conversion of Compounds to AA by Resting Cells of Mutant Strains of *Prototheca* of Varying Abilities to Synthesize AA

Strain	Absolute AA, mg/L					
	Buffer	Glucose	L-galactose	L-gal-γ-lact.	Mannose	Fructose
EMS13-4	53	97	191	173	139	ND
UV127-10	45	140	213	140	128	143
SP2-3	19	19	204	146	24	27
NA21-14	61	80	147	158	118	115
UV82-21	15	16	183	175	18	17
UV213-1	16	15	170	135	17	16
UV218-1	16	18	136	176	19	21
UV244-1	16	16	164	162	16	16
UV244-15	26	77	30	21	94	89
UV244-16	28	64	53	53	53	66

ND = Not Determined

These data suggest that the mutational blocks in those strains which convert fructose and mannose to AA poorly are before ("upstream" from) L-galactose and L-galactono-γ-lactone in the pathway.

Example 6

The following example shows that magnesium inhibits early steps in the production of AA.

To address the question of whether magnesium actually inhibits AA synthesis, strain NA45-3 (ATCC 209681) was grown in magnesium (Mg)-limited and Mg-sufficient medium. ATCC No. 209681, identified as *Protothecа moriformis* NA45-3 (Source:

repeated mutagenesis of ATCC No. 75669; Eucaryotic alga. Division Chlorophyta, Class Chlorophyceae, Order Chlorococcales), was deposited on March 13, 1998, with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. Cells from both cultures were harvested and resuspended in the cell-free supernate from the Mg-limited culture, and to half of each cell suspension additional magnesium was added in order to bring the level in the suspension to the Mg-sufficient level. The four conditions under which assays were run were as follows.

TABLE 10

Conditions Used to Test the Effect of Magnesium on AA Production

Condition	Magnesium concentration, g/L, during:	
	Growth	Assay
1Mg>1Mg	0.02	0.02
1Mg>10Mg	0.02	0.2
10Mg>1Mg	0.2	0.02
10Mg>10Mg	0.2	0.2

Substrates previously shown to lead to the formation of AA, namely D-glucose, D-glucosone, D-fructose. D-galactose, D-mannose, and L-galactono-γ-lactone, were added at 20 g/L to the four cell suspensions. Accumulation of AA after 24 hours was measured and compared to a control in which no substrate was added. The results of this study are shown graphically in Fig. 4.

When cells growing under magnesium-limited conditions were incubated with substrates in low-magnesium broth (1Mg>1Mg condition), all showed significant and similar accumulation of AA over the control condition. When the same cells were incubated in high magnesium broth (1Mg>10Mg condition), the accumulation of AA was reduced about 40% for all substrates except D-mannose and L-galactono-γ-lactone, suggesting that 1) the rate-limiting step in the conversion of D-glucose, D-glucosone, D-fructose, and D-galactose to AA is inhibited by magnesium or 2) magnesium stimulates an enzyme which results in the conversion of these compounds to some other compound(s), reducing the amount of substrate available for AA synthesis. On the other

hand, conversion of D-mannose and L-galactono-γ-lactone appeared to be unaffected by the presence of magnesium in the resuspension buffer, indicating that either 1) magnesium-inhibited enzymes are not involved in the conversion of these substrates to AA or 2) D-mannose and L-galactono-γ-lactone enter the pathway far enough downstream from the point where they can be siphoned off by side reactions involving Mg-requiring enzymes.

When cells were grown under magnesium-sufficient conditions, very little AA accumulation from any of the D-sugars was observed, regardless of the level of magnesium in the resuspension broth. Accumulation of AA from L-galactono-γ-lactone, however, was enhanced over that observed when cells are grown in Mg-limited conditions. This suggests that enzymes early in the pathway are repressed under Mg-sufficient conditions. Thus, the D-substrates all behaved similarly, with the exception of the apparent lack of magnesium inhibition of D-mannose conversion to AA. This would suggest that D-mannose enters the AA biosynthetic pathway at a point other than the other D-sugars.

Figs. 2A and 2B represent some of the fates of glucose in plants. The first enzymatic step in this scheme which commits carbon to glycolysis is the conversion of fructose-6-P to fructose-1,6-diP by phosphofructokinase (PFK). This reaction is essentially irreversible, and leads to the well known TCA cycle and oxidative phosphorylation, with concomitant ATP and NADH/NADPH generation. PFK has an absolute requirement for magnesium. If magnesium is limiting, this reaction could slow and eventually stop, blocking the flow of carbon through glycolysis and beyond, and would result in cessation of cell division even in the presence of excess glucose. One would expect fructose-6-P to accumulate under these conditions, fueling AA synthesis by the pathway shown in Figs. 1 and 2.

Example 7

The following example shows the correlation in *Prototheca* between AA production and the activity levels of the enzymes in the AA pathway.

Phosphomannose isomerase (PMI) Assay

PMI activity was first assayed (See Fig. 1). Ten strains representing a range of AA productivities were grown according to the standard protocol to measure AA-synthesizing ability. Cells were harvested 96 hours into magnesium-limited incubation, washed and resuspended in buffer containing 50 mM Tris/10 mM $MgCl_2$, pH 7.5. The suspended cells were broken in a French press, spun at 30,000 x g for 30 minutes, and desalted through Sephadex G-25 (Pharmacia PD-10 columns). Reactions were carried out in the reverse direction by adding various volumes of extracts to solutions of Tris/Mg buffer containing 0.15 U phosphoglucose isomerase (EC 5.3.1.9), 0.5 U glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and 1.0 mM NADP. Reactions were initiated by addition of 3 mM (final) mannose-6-phosphate. Final reaction volume was 1.0 mL. All components were dissolved in Tris/Mg buffer. Activities were taken as the change in A_{340}/min. From these activities was subtracted the activities measured in identical reaction mixtures lacking the M-6-P substrate. Specific activities were calculated by normalizing the activities for protein concentration in the reactions. Protein in the original extracts was determined by the method of Bradford, using a kit from Bio-Rad Laboratories (Hercules, CA). All enzymes and nucleotides were purchased from Sigma Chemical Co. (St. Louis, MO).

Phosphomannomutase (PMM) Assay

Phosphomannomutase was measured in a similar manner in the same strains, but these assay reaction mixtures also contained 0.25 mM glucose-1,6-diphosphate, 0.5 U commercially available PMI, and the reactions were started with the addition of 3.0 mM (final) mannose-1-phosphate rather than mannose-6-phosphate.

Phosphofructokinase (PFK) Assay

To shed light on the possibility that the enhancement of AA concentration in cultures which were limited for magnesium was due to a diversion of carbon from normal metabolism by a reduced activity of the first committed step in glycolysis (PFK) the strains were also assayed to confirm the presence of this enzyme activity. Cells were cultured, washed and broken as above. Extracts were centrifuged at 100,000 x g for 90 min before

desalting. Reactions were carried out in the forward direction by adding various volumes of extracts to solutions of Tris/Mg buffer containing 1.5 mM dithiothreitol, 0.86 U aldolase (EC 4.1.2.13), 1.4 U α-glycerophosphate dehydrogenase (EC 1.1.1.8), 14 U triosephosphate isomerase (EC 5.3.1.1), 0.11 mM NADH, and 1.0 mM ATP. Reactions were initiated by addition of 5 mM (final) fructose-6-phosphate. Final reaction volume was 1.0 mL. All components were dissolved in Tris/Mg buffer. Activities were taken as the change in A_{340}/min. From these activities were subtracted the activities measured in identical reaction mixtures lacking the F-6-P substrate. Specific activities were calculated by normalizing the activities for protein concentration in the reaction. Protein in the original extracts was determined as above.

GDP-D-mannose pyrophosphorylase (GMP) Assay

These same mutant strains were assayed for the next enzyme in the proposed pathway, GMP. Strains were grown both according to the standard Mg-limiting protocol (harvested 43-48 hours into magnesium-limited incubation) and in standard Mg-sufficient medium (harvesting all cells before glucose depletion). Washed cell pellets were resuspended in 50 mM phosphate buffer, pH 7.0, containing 20% (v/v) glycerol and 0.1 M sodium chloride (3 mL buffer/g wet cells), and broken in a French press. Crude extracts were spun at 15,000 x g for 15 minutes. Reactions were carried out in the forward direction by adding various volumes of extracts to solutions of 50 mM phosphate/4 mM $MgCl_2$ buffer, pH 7.0, containing 1 mM GTP. Reactions were initiated by addition of 1 mM (final) mannose-1-phosphate. Final reaction volume was 0.1 mL. Reaction mixtures were incubated at 30 C for 10 min, filtered through a 0.45 μm PVDF syringe filter, and analyzed for GDP-mannose by HPLC. A Supelcosil SAX1 column (4.6 x 250 mm) was used with a solvent gradient (1 mL/min) of: A - 6 mM potassium phosphate, pH 3.6; B - 500 mM potassium phosphate, pH 4.5. The gradient was: 0-3 min, 100% A; 3-10 min, 79% A; 10-15 min, 29% A. Column temperature was 30 C. Two assays that showed enzyme activity proportional to the amount of protein were averaged. Control no-substrate and no-extract reactions were also run. Specific activity was calculated by normalizing the activity for protein concentration in the reaction. Protein in the original extracts was determined as above.

GDP-D-mannose:GDP-L-galactose Epimerase Assay

Further tests measured the activities of the next enzyme in the proposed pathway, GDP-D-mannose:GDP-L-galactose epimerase. Strains were grown according to the standard protocol, harvested 43-48 hours into magnesium-limited incubation, washed, and resuspended in buffer containing 50 mM MOPS/5 mM EDTA, pH 7.2. Washed pellets were broken in a French press, and spun at 20,000 x g for 20 min. Protein determinations were made as above and a dilution series of each was made, ranging from 0.4 to 2.2 mg protein/mL. 50 µL aliquots of these dilutions were added to 10 µL aliquots of 6.3 mM GDP-D-mannose in which a portion of this substrate was universally labeled with ^{14}C in the mannose moiety. This substrate had an activity of 16 µCi/mL before dilution into the reaction mixture. Reactions were stopped after 10 min by transferring 20 µL of the mixture into microfuge tubes containing 20 µL of 250 mM trifluoroacetic acid (TFA) containing 1.0 g/L each D-mannose and L-galactose. These tubes were sealed and boiled for 10 min, cooled, spun for 60 sec in a Beckman Microfuge E, and 5 µL of each hydrolysate was spotted on 20 x 20 cm plastic-backed EM Science Silica gel 60 thin-layer chromatography plates (#5748/7), with 1 cm lanes created by scoring with a blunt stylus. After drying, plates were twice chromatographed for 2.5 hours in ethyl acetate:isopropanol:water, 65:22.3:12.7 (plates were dried between runs). Spots of free sugars were visualized by spraying dried plates with 0.5% p-anisaldehyde in a 62% ethanolic solution of 0.89 M sulfuric acid and 0.17 mM glacial acetic acid, and heating at 105 C for about 15 min. Spots of L-galactose and D-mannose were cut from the plates and counted in a scintillation counter (Beckman model 2800). For time-zero control counts, 16.7 µL of each extract dilution was added to 23.3 µL of the labeled substrate above, which had been diluted 1:7 with the TFA/mannose/galactose solution.

Table 11 summarizes the results of the five enzyme assays for the strains tested, along with their specific AA formations.

TABLE 11

Specific Enzyme Activities (mU)* of Selected Mutant *Prototheca* Strains

Strain	AA Specific Form, mg/g	PMI	PMM	PFK	GMP		Epimerase
					Mg-limited	Mg-sufficient	
UV164-6	78.4						0.79
EMS13-4	73.7	10.8	69.6	13.5	2.6	6.8	0.78
UV140-1	69.9						0.78
NA45-3	61.4						0.58
UV77-247	44.4						0.52
UV127-10	40.1	11.1	45.8	24.4	4.3	5.9	0.39
UV244-15	24.5	14.3	41.5		3.1	5.3	0.42
NA21-14	23.6	12.1	60.3	47.4	2.4	7.6	0.27
ATCC 75669	21.9						0.28
UV244-16	5.0	16.5	85.6		4.3	5.2	
SP2-3	2.0	17.7	47.0	64.5	2.0	7.5	0.03
UV218-1	0.4	15.9	72.1		2.7	7.0	0.83
UV213-1	0.1	19.7	47.7	32.6	3.2	6.7	0.60
UV82-21	0.0	14.6	70.6	30.4	4.1	7.5	0.15
UV244-1	0.0	18.2	51.1		5.5	12	0.15

* Units: PMI and PMM, nmoles NADP reduced per min/mg protein; PFK, nmoles NADH oxidized per min/mg protein; GMP, nmoles GDP-D-mannose formed per min/mg protein; epimerase, nmoles GDP-L-galactose formed per min/mg protein.

The only enzyme which showed a strong correlation between activity and the ability to synthesize AA was the GDP-D-mannose:GDP-L-galactose epimerase. This correlation is depicted in Fig. 5. All of the strains which produced measurable amounts of AA had measurable amounts of epimerase activity. The converse was not true: four of the strains which synthesize little or no AA had significant epimerase activities. These strains are candidates for having mutations which affect enzymatic steps downstream from the epimerase. Since all of the strains tested can synthesize AA from L-galactose and L-galactono-γ-lactone (see Examples 4 and 5), the genetic lesion(s) in these four mutants must lie between GDP-L-galactose and free L-galactose.

Example 8

The next example shows the relationship between GDP-D-mannose:GDP-L-galactose epimerase activity and the degree of magnesium limitation in two strains, the original unmutagenized parent strain ATCC 75669, and one of the best AA producers, EMS13-4 (ATCC _____).

Four flasks of each strain were grown according to the standard protocol. One culture of each was harvested 24 hours into magnesium-limited incubation, and every 24 hours thereafter for a total of four days. One flask of each strain was also harvested 24 hours into magnesium sufficient incubation. All cultures had glucose remaining when harvested. Fig. 6 shows graphically the AA productivity and epimerase activity in EMS13-4 and ATCC 75669 as the cultures became Mg-limited. Epimerase activity in EMS13-4 was significantly greater than that in ATCC 75669 at all time points. There was also a concurrent rapid rise in both AA productivity and epimerase activity in EMS13-4 as the cultures became increasingly Mg-limited. While there was a moderate increase in AA productivity in ATCC 75669 as Mg became more limiting, there was no effect on epimerase activity.

Example 9

The following example shows the results of epimerase assays performed with extracts of two *E. coli* strains into which were cloned the *E. coli* gene for GDP-4-keto-6-deoxy-D-mannose epimerase/reductase.

The *E. coli* K12 *wca* gene cluster is responsible for cholanic acid production; *wcaG* encodes a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase.

The *E. coli wcaG* sequence (nucleotides 4 through 966 of SEQ ID NO:3) was amplified by PCR from *E. coli* W3110 genomic DNA using primers WG EcoRI 5 (5' TAGAATTCAGTAAACAACGAGTTTTTATTGCTGG 3'; SEQ ID NO:12) and WG XhoI 3 (5' AACTCGAGTTACCCCCAAAGCGGTCTTGATTC 3'; SEQ ID NO:13). The 973-bp PCR product was ligated into the vector pPCR-Script SK(+) (Stratagene, LaJolla, CA). The 973-bp ExoRII/XhoI fragment was moved from this plasmid into the ExoRII/XhoI sites of pGEX-5X-1 (Amersham Pharmacia Biotech, Piscataway, NJ), creating plasmid pSW67-1. Plasmid pGEX-5X-1 is a GST gene fusion vector which adds a 26-kDa GST moiety onto the N-terminal end of the protein of interest. *E. coli* BL21(DE3) was transformed with pSW67-1 and pGEX-5X-1, resulting in strains BL21(DE3)/pSW67-1 and BL21(DE3)/pGEX-5X-1.

The *E. coli wcaG* sequence (nucleotides 1 through 966 of SEQ ID NO:3) was also amplified by PCR from *E. coli* W3110 genomic DNA using primers WG EcoRI 5-2 (5' CTGGAGTCGAATTCATGAGTAAACAACGAG 3'; SEQ ID NO:14) and WG PstI 3

(5' AACTGCAGTTACCCCCGAAAGCGGTCTTGATTC 3'; SEQ ID NO:15). The 976-bp PCR product was ligated into a pPCR-Script (Stratagene). The 976-bp ExoRII/PstI fragment was moved from this plasmid into the ExoRII/PstI sites of expression vector pKK223-3 (Amersham Pharmacia Biotech), creating plasmid pSW75-2. *E. coli* JM105 was transformed with pKK223-3 and pSW75-2, resulting in strains JM105/pKK223-3 and JM105/pSW75-2.

All six strains were grown in duplicate at 37°C with shaking in 2X YTA medium until an optical density of 0.8-1.0 at 600 nm was reached (about three hours). 2X YTA contains 16 g/L tryptone, 10 g/L yeast extract, 5 g/L sodium chloride and 100 mg/L ampicillin. One of each culture was induced by adding isopropyl β-D-thiogalactopyranoside (IPTG) to 1 mM final concentration. All 12 cultures were incubated for an additional four hours, washed in 0.9% NaCl, and the cells were frozen at -80°C. Prior to pelleting the cells for preparation of extracts, a portion of each culture was used for a plasmid DNA miniprep to confirm the presence of the appropriate plasmids in these strains. A protein preparation of each culture was also run on SDS gels to confirm expression of a protein of the appropriate size where expected. Frozen pellets were thawed, resuspended in 2.5 mL MOPS/EDTA buffer, pH 7.2, broken in a French Press (10,000 psi), spun for 20 min at 20,000 x g, assayed for protein as above and diluted to 0.01, 0.1, 1.0 and 3 mg/mL protein.

Induction of the strain BL21(DE3)/pGEX-5X-1 resulted in high-level expression of a 26-kDa protein indicating the synthesis of the native GST protein. Induction of strain BL21(DE3)/pSW67-1 resulted in high-level expression of a 62-kDa protein, indicating the synthesis of the native GST protein (26K) fused to the *wcaG* gene product (36K). An aliquot of the fusion protein was treated with the protease Factor Xa (New England Biolabs, Beverly, MA), which cleaves near the GST/*wcaG* junction. Induction of the strain JM105/pSW75-2 resulted in high level expression of a 36-kDa protein, indicating the synthesis of the *wcaG* gene product. No such protein was detected in JM105/pKK223-3 (vector only).

Next, it was of interest to test extracts in the standard epimerase assay described in Example 7 to determine if any of the extracts containing the *wcaG* product could bring

about the conversion of GDP-D-mannose to GDP-L-galactose. The extracts to be assayed are:

BL21(DE3) Group

1. BL21(DE3) uninduced
2. BL21(DE3) induced with 1mM IPTG
3. BL21(DE3)/pGEX-5X-1 uninduced
4. BL21(DE3)/pGEX-5X-1 induced with 1mM IPTG
5. BL21(DE3)/pSW67-1 uninduced
6. BL21(DE3)/pSW67-1 induced with 1 mM IPTG; fusion protein intact
7. BL21(DE3)/pSW67-1 induced with 1 mM IPTG; GST moiety cleaved

JM105 Group

1. JM105 uninduced
2. JM105 induced with 1mM IPTG
3. JM105/pKK223-3 uninduced
4. JM105/pKK223-3 induced with 1 mM IPTG
5. JM105/pSW75-2 uninduced
6. JM105/pSW75-2 induced with 1 mM IPTG

Extracts 1 and 7 from the BL21(DE3) group and extracts 1 and 6 from the JM105 group were tested for GDP-D-mannose:GDP-L-galactose epimerase-like activity in a pilot experiment. In this initial experiment, no epimerase activity was detected in any of the extracts. At this time, such a result can be attributed to a number of possibilities. First, it is possible that the *wcaG* gene product is incapable of catalyzing the conversion of GDP-D-mannose to GDP-L-galactose, although this conclusion can not be reached until several other parameters are tested. Second, it is possible that under the assay conditions which are satisfactory to measure activity for the endogenous GDP-D-mannose:GDP-L-galactose epimerase, the *wcaG* gene product does not have GDP-D-mannose:GDP-L-galactose epimerase-like activity. Therefore, alternate conditions should be tested. Additionally, confirmation experiments should be performed to confirm the accuracy of the pilot conditions. Third, although the BL21(DE3) and the JM105 clones produce proteins of the expected size, the constructs have not been sequenced to confirm the proper coding sequence for the *wcaG* gene product and thereby rule out PCR or cloning errors which may render the *wcaG* gene product inactive. Fourth, the protein formed from the cloned sequence is full-length, but inactive, for example, due to incorrect tertiary structure (folding). Fifth, the gene is overexpressed, resulting in accumulation of insoluble and inactive protein products (inclusion bodies). Future experiments will attempt to

determine whether the constructs have or can be induced to have the ability to catalyze the conversion of GDP-D-mannose to GDP-L-galactose, and to use the sequences to isolate the endogenous GDP-D-mannose:GDP-L-galactose epimerase.

Table 12 provides the atomic coordinates for Brookhaven Protein Data Bank Accession Code 1bws:

TABLE 12

```
HEADER    EPIMERASE/REDUCTASE                     27-SEP-98   1BWS
TITLE     CRYSTAL STRUCTURE OF GDP-4-KETO-6-DEOXY-D-MANNOSE
TITLE    2 EPIMERASE/REDUCTASE FROM ESCHERICHIA COLI A KEY ENZYME IN
TITLE    3 THE BIOSYNTHESIS OF GDP-L-FUCOSE
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COMPND    2 MOLECULE: GDP-4-KETO-6-DEOXY-D-MANNOSE EPIMERASE/REDUCTASE;
COMPND    3 CHAIN: A;
COMPND    4 ENGINEERED: YES;
COMPND    5 BIOLOGICAL_UNIT: HOMODIMER
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SOURCE    3 EXPRESSION_SYSTEM: ESCHERICHIA COLI
KEYWDS    EPIMERASE/REDUCTASE, GDP-L-FUCOSE BIOSYNTHESIS
EXPDTA    X-RAY DIFFRACTION
AUTHOR    DE M.RIZZITONETTIFLORA
REVDAT    1   13-JAN-99 1BWS    0
JRNL        AUTH   DE D.RIZZITONETTIVIGEVANISTURLABISSOFLORA
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JRNL        TITL 2 FROM ESCHERICHIA COLI, A KEY ENZYME IN THE
JRNL        TITL 3 BIOSYNTHESIS OF GDP-L-FUCOSE, DISPLAYS THE
JRNL        TITL 4 STRUCTURAL CHARACTERISTICS OF THE RED PROTEIN
JRNL        TITL 5 HOMOLOGY SUPERFAMILY
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JRNL        REFN                                     9999
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REMARK 290
REMARK 290      WHERE NNN -> OPERATOR NUMBER
REMARK 290            MMM -> TRANSLATION VECTOR
REMARK 290
REMARK 290 CRYSTALLOGRAPHIC SYMMETRY TRANSFORMATIONS
REMARK 290 THE FOLLOWING TRANSFORMATIONS OPERATE ON THE ATOM/HETATM
REMARK 290 RECORDS IN THIS ENTRY TO PRODUCE CRYSTALLOGRAPHICALLY
REMARK 290 RELATED MOLECULES.
REMARK 290   SMTRY1   1  1.000000  0.000000  0.000000        0.00000
REMARK 290   SMTRY2   1  0.000000  1.000000  0.000000        0.00000
REMARK 290   SMTRY3   1  0.000000  0.000000  1.000000        0.00000
REMARK 290   SMTRY1   2 -0.500045 -0.865974  0.000000        0.00000
REMARK 290   SMTRY2   2  0.866077 -0.499955  0.000000        0.00000
REMARK 290   SMTRY3   2  0.000000  0.000000  1.000000       50.58553
REMARK 290   SMTRY1   3 -0.499955  0.865974  0.000000        0.00000
REMARK 290   SMTRY2   3 -0.866077 -0.500045  0.000000        0.00000
REMARK 290   SMTRY3   3  0.000000  0.000000  1.000000       25.29276
REMARK 290   SMTRY1   4 -0.500045  0.865922  0.000000        0.00000
REMARK 290   SMTRY2   4  0.866077  0.500045  0.000000        0.00000
REMARK 290   SMTRY3   4  0.000000  0.000000 -1.000000        0.00000
REMARK 290   SMTRY1   5  1.000000  0.000104  0.000000        0.00000
REMARK 290   SMTRY2   5  0.000000 -1.000000  0.000000        0.00000
REMARK 290   SMTRY3   5  0.000000  0.000000 -1.000000       25.29276
REMARK 290   SMTRY1   6 -0.499955 -0.866026  0.000000        0.00000
REMARK 290   SMTRY2   6 -0.866077  0.499955  0.000000        0.00000
REMARK 290   SMTRY3   6  0.000000  0.000000 -1.000000       50.58553
REMARK 290
REMARK 290 REMARK: NULL
REMARK 465
REMARK 465 MISSING RESIDUES
REMARK 465 THE FOLLOWING RESIDUES WERE NOT LOCATED IN THE
REMARK 465 EXPERIMENT. (M=MODEL NUMBER; RES=RESIDUE NAME; C=CHAIN
REMARK 465 IDENTIFIER; SSSEQ=SEQUENCE NUMBER; I=INSERTION CODE):
REMARK 465
REMARK 465   M RES C SSSEQI
REMARK 465     MET A     1
REMARK 465     SER A     2
REMARK 465     ASP A   317
REMARK 465     ARG A   318
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REMARK 465     PHE A   319
REMARK 465     ARG A   320
REMARK 465     GLY A   321
REMARK 800
REMARK 800 SITE
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
REMARK 800 SITE_IDENTIFIER: CAT
REMARK 800 SITE_DESCRIPTION:
REMARK 800 CATALYTIC RESIDUE
REMARK 800
DBREF  1BWS A    3   316  SWS    P32055   FCL_ECOLI
SEQRES   1 A  321  MET SER LYS GLN ARG VAL PHE ILE ALA GLY HIS ARG GLY
SEQRES   2 A  321  MET VAL GLY SER ALA ILE ARG ARG GLN LEU GLU GLN ARG
SEQRES   3 A  321  GLY ASP VAL GLU LEU VAL LEU ARG THR ARG ASP GLU LEU
SEQRES   4 A  321  ASN LEU LEU ASP SER ARG ALA VAL HIS ASP PHE PHE ALA
SEQRES   5 A  321  SER GLU ARG ILE ASP GLN VAL TYR LEU ALA ALA ALA LYS
SEQRES   6 A  321  VAL GLY GLY ILE VAL ALA ASN ASN THR TYR PRO ALA ASP
SEQRES   7 A  321  PHE ILE TYR GLN ASN MET MET ILE GLU SER ASN ILE ILE
SEQRES   8 A  321  HIS ALA ALA HIS GLN ASN ASP VAL ASN LYS LEU LEU PHE
SEQRES   9 A  321  LEU GLY SER SER CYS ILE TYR PRO LYS LEU ALA LYS GLN
SEQRES  10 A  321  PRO MET ALA GLU SER GLU LEU LEU GLN GLY THR LEU GLU
SEQRES  11 A  321  PRO THR ASN GLU PRO TYR ALA ILE ALA LYS ILE ALA GLY
SEQRES  12 A  321  ILE LYS LEU CYS GLU SER TYR ASN ARG GLN TYR GLY ARG
SEQRES  13 A  321  ASP TYR ARG SER VAL MET PRO THR ASN LEU TYR GLY PRO
SEQRES  14 A  321  HIS ASP ASN PHE HIS PRO SER ASN SER HIS VAL ILE PRO
SEQRES  15 A  321  ALA LEU LEU ARG ARG PHE HIS GLU ALA THR ALA GLN ASN
SEQRES  16 A  321  ALA PRO ASP VAL VAL VAL TRP GLY SER GLY THR PRO MET
SEQRES  17 A  321  ARG GLU PHE LEU HIS VAL ASP ASP MET ALA ALA ALA SER
SEQRES  18 A  321  ILE HIS VAL MET GLU LEU ALA HIS GLU VAL TRP LEU GLU
SEQRES  19 A  321  ASN THR GLN PRO MET LEU SER HIS ILE ASN VAL GLY THR
SEQRES  20 A  321  GLY VAL ASP CYS THR ILE ARG ASP VAL ALA GLN THR ILE
SEQRES  21 A  321  ALA LYS VAL VAL GLY TYR LYS GLY ARG VAL VAL PHE ASP
SEQRES  22 A  321  ALA SER LYS PRO ASP GLY THR PRO ARG LYS LEU LEU ASP
SEQRES  23 A  321  VAL THR ARG LEU HIS GLN LEU GLY TRP TYR HIS GLU ILE
SEQRES  24 A  321  SER LEU GLU ALA GLY LEU ALA SER THR TYR GLN TRP PHE
SEQRES  25 A  321  LEU GLU ASN GLN ASP ARG PHE ARG GLY
HET    NDP     1       0
HETNAM     NDP NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE
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HETSYN     NDP NADP
FORMUL   2  NDP    C21 H23 N7 O17 P3 3-
FORMUL   3  HOH   *109(H2 O1)
HELIX    1   1 MET A   14  GLN A   25  1                                  12
HELIX    2   2 SER A   44  GLU A   54  1                                  11
HELIX    3   3 ILE A   69  THR A   74  1                                   6
HELIX    4   4 PRO A   76  ASN A   97  1                                  22
HELIX    5   5 SER A  108  ILE A  110  5                                   3
HELIX    6   6 GLU A  121  GLU A  123  5                                   3
HELIX    7   7 GLU A  134  TYR A  154  1                                  21
HELIX    8   8 VAL A  180  ALA A  193  1                                  14
HELIX    9   9 VAL A  214  GLU A  226  1                                  13
HELIX   10  10 HIS A  229  GLU A  234  1                                   6
HELIX   11  11 ILE A  253  VAL A  264  1                                  12
HELIX   12  12 THR A  288  GLN A  292  1                                   5
HELIX   13  13 LEU A  301  GLU A  314  1                                  14
SHEET    1   A 6 VAL A  29  VAL A  32  0
SHEET    2   A 6 GLN A   4  ALA A   9  1  N  GLN A   4   O  GLU A  30
SHEET    3   A 6 GLN A  58  LEU A  61  1  N  GLN A  58   O  PHE A   7
SHEET    4   A 6 LYS A 101  LEU A 105  1  N  LYS A 101   O  VAL A  59
SHEET    5   A 6 ASP A 157  PRO A 163  1  N  ASP A 157   O  LEU A 102
SHEET    6   A 6 ILE A 243  VAL A 245  1  N  ILE A 243   O  MET A 162
SHEET    1   B 2 ASN A 165  TYR A 167  0
SHEET    2   B 2 PHE A 211  HIS A 213  1  N  LEU A 212   O  ASN A 165
SHEET    1   C 2 ASP A 198  TRP A 202  0
SHEET    2   C 2 ARG A 269  ASP A 273  1  N  ARG A 269   O  VAL A 199
SITE     1 CAT  1 TYR  136
SITE     2 CAT  1 LYS  140
SITE     3 CAT  1 SER  107
CRYST1  104.200  104.200   75.880  90.00  90.00 120.00 P 32 2 1      6
ORIGX1      1.000000  0.000000  0.000000        0.00000
ORIGX2      0.000000  1.000000  0.000000        0.00000
ORIGX3      0.000000  0.000000  1.000000        0.00000
SCALE1      0.009597  0.005541  0.000000        0.00000
SCALE2      0.000000  0.011081  0.000000        0.00000
SCALE3      0.000000  0.000000  0.013179        0.00000
HETATM   1  O   HOH     1      55.652 -16.806  22.535  1.00  8.73           O
HETATM   2  O   HOH     3      58.494 -10.639  18.740  1.00 13.17           O
HETATM   3  O   HOH     4      58.230 -11.715  27.770  1.00 19.07           O
HETATM   4  O   HOH     5      57.252  -3.759  30.107  1.00 11.21           O
HETATM   5  O   HOH     6      58.298 -10.011  25.527  1.00 15.74           O
HETATM   6  O   HOH     7      49.321   6.583  38.815  1.00 19.33           O
HETATM   7  O   HOH     8      53.785  -4.262  22.464  1.00 10.94           O
HETATM   8  O   HOH    10      74.652   2.888   9.141  1.00 17.80           O
HETATM   9  O   HOH    11      49.761   0.826  32.896  1.00 22.02           O
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HETATM   10  O   HOH    12      55.530 -11.162  28.526  1.00 11.39           O
HETATM   11  O   HOH    13      75.027   7.034  27.353  1.00 16.30           O
HETATM   12  O   HOH    14      49.994  -2.314  11.032  1.00 21.33           O
HETATM   13  O   HOH    15      61.323  -8.959  29.657  1.00 22.84           O
HETATM   14  O   HOH    16      61.029 -11.560  29.131  1.00 21.24           O
HETATM   15  O   HOH    17      50.684   5.881  10.130  1.00 15.88           O
HETATM   16  O   HOH    18      64.506  -6.302  32.989  1.00 21.05           O
HETATM   17  O   HOH    19      57.856 -16.398  25.085  1.00 22.86           O
HETATM   18  O   HOH    20      38.979  26.536  19.070  1.00 21.08           O
HETATM   19  O   HOH    21      38.042  33.487  21.909  1.00 19.01           O
HETATM   20  O   HOH    24      38.172  35.775  20.827  1.00 33.46           O
HETATM   21  O   HOH    25      70.916 -11.128  15.244  1.00 31.37           O
HETATM   22  O   HOH    26      54.205  19.360  28.396  1.00 35.76           O
HETATM   23  O   HOH    27      50.436   2.654  16.783  1.00 12.25           O
HETATM   24  O   HOH    28      69.692  19.108  38.979  1.00 49.77           O
HETATM   25  O   HOH    29      56.432  -8.877  19.303  1.00 22.52           O
HETATM   26  O   HOH    30      60.832   3.415  42.349  1.00 17.39           O
HETATM   27  O   HOH    31      53.889 -12.706  29.764  1.00 22.40           O
HETATM   28  O   HOH    32      37.887  26.373  28.058  1.00 18.09           O
HETATM   29  O   HOH    33      49.201  11.173  26.867  1.00 33.95           O
HETATM   30  O   HOH    34      46.762  -0.278  31.394  1.00 20.63           O
HETATM   31  O   HOH    35      41.731  27.568  43.302  1.00 27.39           O
HETATM   32  O   HOH    36      66.827  11.202  28.929  1.00 13.23           O
HETATM   33  O   HOH    37      46.834  14.396  40.819  1.00 46.02           O
HETATM   34  O   HOH    38      61.342   1.064  43.868  1.00 26.68           O
HETATM   35  O   HOH    42      70.597  16.422  37.837  1.00 19.26           O
HETATM   36  O   HOH    44      72.275  -9.089  33.407  1.00 22.11           O
HETATM   37  O   HOH    45      42.685  34.461  33.955  1.00 17.32           O
HETATM   38  O   HOH    46      53.480  13.394  38.364  1.00 20.19           O
HETATM   39  O   HOH    47      56.085  21.757  44.744  1.00 33.50           O
HETATM   40  O   HOH    48      35.741  32.691  23.517  1.00 19.49           O
HETATM   41  O   HOH    49      40.458  36.700  34.312  1.00 34.53           O
HETATM   42  O   HOH    50      75.440   7.267  29.948  1.00 18.07           O
HETATM   43  O   HOH    51      47.476  18.347  20.851  1.00 34.16           O
HETATM   44  O   HOH    53      52.837 -16.344  19.587  1.00 25.92           O
HETATM   45  O   HOH    55      46.415   9.073  20.108  1.00 31.91           O
HETATM   46  O   HOH    57      45.912  35.170  36.133  1.00 35.55           O
HETATM   47  O   HOH    58      60.247  -2.880  41.919  1.00 16.85           O
HETATM   48  O   HOH    60      64.974   6.086  24.501  1.00 32.16           O
HETATM   49  O   HOH    61      52.103   4.683   4.978  1.00 35.72           O
HETATM   50  O   HOH    62      50.888  40.154  36.463  1.00 38.35           O
HETATM   51  O   HOH    63      44.373  31.233  37.336  1.00 20.07           O
HETATM   52  O   HOH    64      57.280  27.757  42.451  1.00 21.74           O
HETATM   53  O   HOH    65      58.409  23.769  45.517  1.00 58.42           O
HETATM   54  O   HOH    66      68.690 -11.764  35.335  1.00 57.07           O
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HETATM 55 O HOH 67 42.746 25.153 23.465 1.00 27.05 O
HETATM 56 O HOH 68 53.638 -16.457 32.292 1.00 31.71 O
HETATM 57 O HOH 69 33.390 41.716 31.408 1.00 29.92 O
HETATM 58 O HOH 70 57.768 17.897 42.434 1.00 25.75 O
HETATM 59 O HOH 71 75.647 9.164 11.766 1.00 35.13 O
HETATM 60 O HOH 72 62.032 33.292 44.749 1.00 46.18 O
HETATM 61 O HOH 73 47.310 14.312 34.285 1.00 31.18 O
HETATM 62 O HOH 74 79.660 -3.947 15.913 1.00 34.63 O
HETATM 63 O HOH 75 46.929 5.343 4.550 1.00 23.14 O
HETATM 64 O HOH 76 73.475 12.039 28.412 1.00 27.26 O
HETATM 65 O HOH 77 46.297 -6.982 30.032 1.00 43.41 O
HETATM 66 O HOH 78 68.528 -3.422 40.869 1.00 38.47 O
HETATM 67 O HOH 79 62.080 -1.448 42.803 1.00 24.60 O
HETATM 68 O HOH 80 65.330 18.150 40.726 1.00 41.00 O
HETATM 69 O HOH 81 51.775 16.128 37.607 1.00 25.11 O
HETATM 70 O HOH 83 54.266 28.682 43.313 1.00 27.61 O
HETATM 71 O HOH 85 73.291 -15.479 20.603 1.00 37.54 O
HETATM 72 O HOH 86 34.760 21.479 28.544 1.00 43.87 O
HETATM 73 O HOH 87 37.326 24.131 29.677 1.00 24.47 O
HETATM 74 O HOH 88 65.168 20.148 6.735 1.00 26.10 O
HETATM 75 O HOH 89 59.196 12.089 13.630 1.00 25.24 O
HETATM 76 O HOH 91 66.576 -6.235 40.279 1.00 43.11 O
HETATM 77 O HOH 93 37.339 29.394 25.515 1.00 27.56 O
HETATM 78 O HOH 94 52.339 -17.014 42.271 1.00 48.96 O
HETATM 79 O HOH 95 40.511 32.927 31.717 1.00 22.46 O
HETATM 80 O HOH 96 78.580 13.121 34.138 1.00 27.98 O
HETATM 81 O HOH 97 65.090 15.704 34.876 1.00 18.96 O
HETATM 82 O HOH 99 84.562 2.951 27.181 1.00 35.92 O
HETATM 83 O HOH 100 50.386 9.761 9.646 1.00 23.18 O
HETATM 84 O HOH 101 67.649 -0.851 38.769 1.00 24.99 O
HETATM 85 O HOH 102 44.001 4.293 34.315 1.00 31.13 O
HETATM 86 O HOH 103 59.386 -5.071 26.211 1.00 29.10 O
HETATM 87 O HOH 104 77.364 4.745 41.506 1.00 35.32 O
HETATM 88 O HOH 105 59.034 21.201 32.414 1.00 23.43 O
HETATM 89 O HOH 106 42.463 34.698 14.327 1.00 38.86 O
HETATM 90 O HOH 107 70.217 14.292 20.864 1.00 42.39 O
HETATM 91 O HOH 108 76.999 8.130 25.862 1.00 32.91 O
HETATM 92 O HOH 109 49.766 29.937 22.173 1.00 42.52 O
HETATM 93 O HOH 110 72.473 13.536 38.823 1.00 33.32 O
HETATM 94 O HOH 111 64.328 -12.084 38.608 1.00 37.99 O
HETATM 95 O HOH 112 60.161 16.382 42.682 1.00 35.68 O
HETATM 96 O HOH 113 47.602 13.639 27.016 1.00 26.01 O
HETATM 97 O HOH 115 64.606 11.644 40.107 1.00 30.33 O
HETATM 98 O HOH 116 61.231 -15.137 27.255 1.00 38.76 O
HETATM 99 O HOH 117 65.324 -11.223 35.098 1.00 30.45 O

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HETATM  100  O   HOH   119      56.602  17.219  44.932  1.00 36.53           O
HETATM  101  O   HOH   120      37.564  19.860  23.135  1.00 31.27           O
HETATM  102  O   HOH   121      64.845   5.057  21.132  1.00 45.57           O
HETATM  103  O   HOH   123      63.391  16.801  26.898  1.00 38.46           O
HETATM  104  O   HOH   124      42.567   6.134  32.635  1.00 31.56           O
HETATM  105  O   HOH   125      72.485  13.236  35.059  1.00 29.61           O
HETATM  106  O   HOH   126      65.229   3.650  44.032  1.00 36.86           O
HETATM  107  O   HOH   127      37.089   7.148  31.083  1.00 39.58           O
HETATM  108  O   HOH   128      73.327  10.546  12.123  1.00 34.97           O
HETATM  109  O   HOH   129      74.450  10.299  26.598  1.00 30.80           O
HETATM  110 AO5* NDP A   1      67.524  13.055  26.692  1.00 36.42           O
HETATM  111 AC5* NDP A   1      68.089  12.297  25.614  1.00  9.30           C
HETATM  112 AC4* NDP A   1      69.601  12.124  25.858  1.00 27.73           C
HETATM  113 AO4* NDP A   1      70.193  11.258  24.848  1.00 22.87           O
HETATM  114 AC3* NDP A   1      70.484  13.390  25.873  1.00 17.83           C
HETATM  115 AO3* NDP A   1      71.192  13.436  27.066  1.00 16.11           O
HETATM  116 AC2* NDP A   1      71.373  13.220  24.626  1.00 11.46           C
HETATM  117 AO2* NDP A   1      72.623  13.886  24.655  1.00 31.96           O
HETATM  118 AC1* NDP A   1      71.510  11.702  24.656  1.00 19.02           C
HETATM  119  O3   NDP A   1      65.336  13.590  26.129  1.00 20.59           O
HETATM  120 NO5* NDP A   1      63.536  11.943  26.448  1.00 28.99           O
HETATM  121 NC5* NDP A   1      64.328  10.843  25.957  1.00 24.89           C
HETATM  122 NC4* NDP A   1      63.467   9.646  25.686  1.00 31.79           C
HETATM  123 NO4* NDP A   1      62.837   9.337  26.908  1.00 28.82           O
HETATM  124 NC3* NDP A   1      62.340   9.837  24.665  1.00 11.50           C
HETATM  125 NO3* NDP A   1      62.891   9.402  23.461  1.00 28.60           O
HETATM  126 NC2* NDP A   1      61.152   8.996  25.138  1.00 28.11           C
HETATM  127 NO2* NDP A   1      60.881   7.662  24.715  1.00 24.30           O
HETATM  128 NC1* NDP A   1      61.547   8.875  26.580  1.00 35.35           C
HETATM  129 AP2* NDP A   1      73.104  15.069  23.823  1.00 32.96           P
HETATM  130 AOP1 NDP A   1      74.500  15.308  24.308  1.00 37.84           O
HETATM  131 AOP2 NDP A   1      72.797  14.925  22.348  1.00 36.66           O
HETATM  132 AOP3 NDP A   1      72.163  16.217  23.958  1.00 31.97           O
HETATM  133  AP  NDP A   1      66.660  14.257  26.393  1.00 26.17          XX
HETATM  134  AO1 NDP A   1      66.886  14.795  25.047  1.00 15.31          XX
HETATM  135  AO2 NDP A   1      66.439  15.207  27.521  1.00 34.39          XX
HETATM  136  AN9 NDP A   1      71.820  11.224  23.353  1.00 13.63          XX
HETATM  137  AC8 NDP A   1      71.104  11.316  22.200  1.00 12.41          XX
HETATM  138  AN7 NDP A   1      71.758  10.835  21.161  1.00 15.71          XX
HETATM  139  AC5 NDP A   1      72.933  10.313  21.710  1.00 16.17          XX
HETATM  140  AC6 NDP A   1      74.053   9.657  21.140  1.00 31.35          XX
HETATM  141  AN6 NDP A   1      74.165   9.464  19.819  1.00 12.59          XX
HETATM  142  AN1 NDP A   1      75.078   9.280  21.942  1.00 17.56          XX
HETATM  143  AC2 NDP A   1      74.971   9.578  23.251  1.00 15.44          XX
HETATM  144  AN3 NDP A   1      74.027  10.302  23.889  1.00 24.82          XX
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HETATM  145  AC4 NDP A   1      73.036  10.653  23.047  1.00 17.48           XX
HETATM  146  NP  NDP A   1      64.183  13.106  27.191  1.00 25.47            N
HETATM  147  NO1 NDP A   1      63.142  14.169  27.253  1.00 28.69            N
HETATM  148  NO2 NDP A   1      64.837  12.643  28.492  1.00 24.32            N
HETATM  149  NN1 NDP A   1      60.598   9.775  27.109  1.00 23.63            N
HETATM  150  NC2 NDP A   1      60.143  10.905  26.442-99.00 78.36            N
HETATM  151  NC3 NDP A   1      59.070  11.648  27.007-99.00100.00            N
HETATM  152  NC7 NDP A   1      58.497  13.017  26.528-99.00100.00            N
HETATM  153  NO7 NDP A   1      59.358  13.703  25.972-99.00100.00            N
HETATM  154  NN7 NDP A   1      57.207  13.400  26.912-99.00 84.38            N
HETATM  155  NC4 NDP A   1      58.442  11.146  28.137-99.00100.00            N
HETATM  156  NC5 NDP A   1      58.912   9.963  28.754-99.00100.00            N
HETATM  157  NC6 NDP A   1      59.951   9.266  28.147-99.00100.00            N
ATOM    158  N   LYS A   3      76.227  -5.632  44.315  1.00 61.49            N
ATOM    159  CA  LYS A   3      76.152  -4.302  43.684  1.00 58.00            C
ATOM    160  C   LYS A   3      75.985  -4.421  42.171  1.00 52.79            C
ATOM    161  O   LYS A   3      76.921  -4.737  41.419  1.00 44.76            O
ATOM    162  CB  LYS A   3      77.359  -3.417  44.030  1.00 59.74            C
ATOM    163  CG  LYS A   3      77.011  -1.944  44.314  1.00 50.87            C
ATOM    164  CD  LYS A   3      78.208  -1.161  44.894  1.00 61.21            C
ATOM    165  CE  LYS A   3      77.855  -0.377  46.186  1.00100.00            C
ATOM    166  NZ  LYS A   3      78.857  -0.401  47.343  1.00 70.61            N
ATOM    167  N   GLN A   4      74.746  -4.242  41.747  1.00 45.15            N
ATOM    168  CA  GLN A   4      74.408  -4.326  40.347  1.00 37.18            C
ATOM    169  C   GLN A   4      74.983  -3.166  39.561  1.00 34.93            C
ATOM    170  O   GLN A   4      75.127  -2.050  40.087  1.00 28.48            O
ATOM    171  CB  GLN A   4      72.915  -4.445  40.221  1.00 34.65            C
ATOM    172  CG  GLN A   4      72.456  -5.854  40.584  1.00 31.82            C
ATOM    173  CD  GLN A   4      72.570  -6.788  39.405  1.00 79.25            C
ATOM    174  OE1 GLN A   4      72.165  -6.452  38.286  1.00100.00            O
ATOM    175  NE2 GLN A   4      73.206  -7.925  39.623  1.00 80.24            N
ATOM    176  N   ARG A   5      75.475  -3.495  38.375  1.00 27.16            N
ATOM    177  CA  ARG A   5      76.146  -2.546  37.483  1.00 39.16            C
ATOM    178  C   ARG A   5      75.191  -2.018  36.433  1.00 38.22            C
ATOM    179  O   ARG A   5      74.938  -2.698  35.438  1.00 32.44            O
ATOM    180  CB  ARG A   5      77.398  -3.163  36.826  1.00 41.76            C
ATOM    181  CG  ARG A   5      78.692  -2.954  37.663  1.00 37.34            C
ATOM    182  CD  ARG A   5      80.015  -3.236  36.876  1.00 32.99            C
ATOM    183  NE  ARG A   5      81.036  -2.203  37.125  1.00 25.71            N
ATOM    184  CZ  ARG A   5      81.617  -1.488  36.169  1.00 32.53            C
ATOM    185  NH1 ARG A   5      81.293  -1.704  34.904  1.00 40.07            N
ATOM    186  NH2 ARG A   5      82.516  -0.551  36.474  1.00100.00            N
ATOM    187  N   VAL A   6      74.743  -0.773  36.659  1.00 32.08            N
ATOM    188  CA  VAL A   6      73.715  -0.082  35.881  1.00 28.89            C
ATOM    189  C   VAL A   6      74.161   1.021  34.897  1.00 29.37            C
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ATOM	190	O	VAL A	6	74.745	2.041	35.274	1.00	22.50	O
ATOM	191	CB	VAL A	6	72.577	0.378	36.813	1.00	23.52	C
ATOM	192	CG1	VAL A	6	71.366	0.960	36.006	1.00	20.29	C
ATOM	193	CG2	VAL A	6	72.108	-0.852	37.644	1.00	18.45	C
ATOM	194	N	PHE A	7	73.948	0.749	33.615	1.00	22.92	N
ATOM	195	CA	PHE A	7	74.267	1.710	32.573	1.00	27.15	C
ATOM	196	C	PHE A	7	72.975	2.423	32.192	1.00	20.24	C
ATOM	197	O	PHE A	7	71.994	1.788	31.815	1.00	20.71	O
ATOM	198	CB	PHE A	7	74.864	1.004	31.374	1.00	18.98	C
ATOM	199	CG	PHE A	7	74.916	1.836	30.115	1.00	21.83	C
ATOM	200	CD1	PHE A	7	75.521	3.087	30.108	1.00	19.36	C
ATOM	201	CD2	PHE A	7	74.483	1.284	28.886	1.00	23.50	C
ATOM	202	CE1	PHE A	7	75.614	3.828	28.902	1.00	27.52	C
ATOM	203	CE2	PHE A	7	74.548	1.996	27.685	1.00	19.33	C
ATOM	204	CZ	PHE A	7	75.128	3.255	27.673	1.00	18.59	C
ATOM	205	N	ILE A	8	72.959	3.727	32.454	1.00	18.75	N
ATOM	206	CA	ILE A	8	71.844	4.588	32.112	1.00	14.25	C
ATOM	207	C	ILE A	8	72.337	5.351	30.909	1.00	11.22	C
ATOM	208	O	ILE A	8	73.259	6.165	30.998	1.00	17.76	O
ATOM	209	CB	ILE A	8	71.507	5.605	33.212	1.00	14.15	C
ATOM	210	CG1	ILE A	8	71.356	4.949	34.582	1.00	8.24	C
ATOM	211	CG2	ILE A	8	70.183	6.342	32.874	1.00	16.85	C
ATOM	212	CD1	ILE A	8	71.091	5.961	35.707	1.00	10.32	C
ATOM	213	N	ALA A	9	71.896	4.906	29.752	1.00	16.42	N
ATOM	214	CA	ALA A	9	72.256	5.559	28.513	1.00	18.74	C
ATOM	215	C	ALA A	9	71.530	6.913	28.511	1.00	28.45	C
ATOM	216	O	ALA A	9	70.411	7.032	29.045	1.00	22.39	O
ATOM	217	CB	ALA A	9	71.808	4.731	27.311	1.00	14.43	C
ATOM	218	N	GLY A	10	72.199	7.922	27.940	1.00	20.06	N
ATOM	219	CA	GLY A	10	71.706	9.284	27.911	1.00	18.62	C
ATOM	220	C	GLY A	10	71.407	9.819	29.305	1.00	16.40	C
ATOM	221	O	GLY A	10	70.379	10.448	29.481	1.00	17.36	O
ATOM	222	N	HIS A	11	72.295	9.581	30.272	1.00	10.32	N
ATOM	223	CA	HIS A	11	72.068	9.966	31.688	1.00	13.90	C
ATOM	224	C	HIS A	11	72.008	11.504	31.916	1.00	21.52	C
ATOM	225	O	HIS A	11	71.700	11.994	32.983	1.00	13.22	O
ATOM	226	CB	HIS A	11	73.153	9.350	32.581	1.00	14.88	C
ATOM	227	CG	HIS A	11	74.502	9.948	32.326	1.00	23.73	C
ATOM	228	ND1	HIS A	11	75.239	9.648	31.197	1.00	24.90	N
ATOM	229	CD2	HIS A	11	75.167	10.952	32.956	1.00	16.35	C
ATOM	230	CE1	HIS A	11	76.317	10.407	31.170	1.00	22.54	C
ATOM	231	NE2	HIS A	11	76.271	11.240	32.197	1.00	17.56	N
ATOM	232	N	ARG A	12	72.310	12.288	30.908	1.00	22.31	N
ATOM	233	CA	ARG A	12	72.147	13.693	31.122	1.00	18.90	C
ATOM	234	C	ARG A	12	70.851	14.244	30.495	1.00	26.34	C

ATOM	235	O	ARG A	12	70.572	15.426	30.604	1.00	25.37	O
ATOM	236	CB	ARG A	12	73.352	14.418	30.587	1.00	25.93	C
ATOM	237	CG	ARG A	12	74.582	13.943	31.279	1.00	53.87	C
ATOM	238	CD	ARG A	12	75.757	14.619	30.699	1.00	32.53	C
ATOM	239	NE	ARG A	12	76.359	15.576	31.605	1.00	69.90	N
ATOM	240	CZ	ARG A	12	76.971	16.675	31.178	1.00	100.00	C
ATOM	241	NH1	ARG A	12	77.001	16.948	29.867	1.00	100.00	N
ATOM	242	NH2	ARG A	12	77.526	17.508	32.056	1.00	100.00	N
ATOM	243	N	GLY A	13	70.078	13.420	29.800	1.00	18.25	N
ATOM	244	CA	GLY A	13	68.802	13.904	29.258	1.00	16.50	C
ATOM	245	C	GLY A	13	67.849	14.144	30.428	1.00	18.88	C
ATOM	246	O	GLY A	13	68.202	13.902	31.624	1.00	14.04	O
ATOM	247	N	MET A	14	66.653	14.632	30.103	1.00	16.00	N
ATOM	248	CA	MET A	14	65.688	14.981	31.128	1.00	13.49	C
ATOM	249	C	MET A	14	65.293	13.760	31.901	1.00	14.02	C
ATOM	250	O	MET A	14	65.408	13.713	33.145	1.00	17.06	O
ATOM	251	CB	MET A	14	64.442	15.605	30.524	1.00	11.57	C
ATOM	252	CG	MET A	14	63.320	15.628	31.559	1.00	20.77	C
ATOM	253	SD	MET A	14	61.926	16.766	31.110	1.00	29.16	S
ATOM	254	CE	MET A	14	62.527	17.108	29.574	1.00	30.68	C
ATOM	255	N	VAL A	15	64.798	12.769	31.158	1.00	25.23	N
ATOM	256	CA	VAL A	15	64.439	11.468	31.738	1.00	20.90	C
ATOM	257	C	VAL A	15	65.654	10.713	32.378	1.00	17.26	C
ATOM	258	O	VAL A	15	65.590	10.239	33.524	1.00	18.41	O
ATOM	259	CB	VAL A	15	63.752	10.550	30.680	1.00	23.25	C
ATOM	260	CG1	VAL A	15	63.330	9.253	31.310	1.00	15.71	C
ATOM	261	CG2	VAL A	15	62.528	11.193	30.183	1.00	13.40	C
ATOM	262	N	GLY A	16	66.784	10.642	31.665	1.00	20.39	N
ATOM	263	CA	GLY A	16	67.941	9.904	32.186	1.00	19.54	C
ATOM	264	C	GLY A	16	68.522	10.432	33.492	1.00	29.29	C
ATOM	265	O	GLY A	16	68.896	9.659	34.434	1.00	16.91	O
ATOM	266	N	SER A	17	68.642	11.755	33.499	1.00	12.53	N
ATOM	267	CA	SER A	17	69.154	12.460	34.650	1.00	21.93	C
ATOM	268	C	SER A	17	68.209	12.214	35.818	1.00	13.35	C
ATOM	269	O	SER A	17	68.677	11.957	36.915	1.00	24.19	O
ATOM	270	CB	SER A	17	69.378	13.942	34.333	1.00	15.52	C
ATOM	271	OG	SER A	17	68.153	14.619	34.372	1.00	22.95	O
ATOM	272	N	ALA A	18	66.896	12.143	35.590	1.00	17.52	N
ATOM	273	CA	ALA A	18	65.991	11.828	36.729	1.00	13.14	C
ATOM	274	C	ALA A	18	66.220	10.393	37.307	1.00	19.29	C
ATOM	275	O	ALA A	18	66.149	10.150	38.522	1.00	16.94	O
ATOM	276	CB	ALA A	18	64.460	12.046	36.334	1.00	14.33	C
ATOM	277	N	ILE A	19	66.484	9.432	36.430	1.00	20.80	N
ATOM	278	CA	ILE A	19	66.705	8.078	36.900	1.00	18.08	C
ATOM	279	C	ILE A	19	67.975	8.090	37.730	1.00	16.09	C

ATOM	280	O	ILE	A	19	68.018	7.530	38.820	1.00 20.73	O
ATOM	281	CB	ILE	A	19	66.804	7.079	35.710	1.00 17.58	C
ATOM	282	CG1	ILE	A	19	65.444	6.812	35.162	1.00 10.09	C
ATOM	283	CG2	ILE	A	19	67.309	5.666	36.133	1.00 21.60	C
ATOM	284	CD1	ILE	A	19	65.528	6.361	33.741	1.00 19.05	C
ATOM	285	N	ARG	A	20	68.984	8.771	37.198	1.00 18.13	N
ATOM	286	CA	ARG	A	20	70.286	8.897	37.836	1.00 20.25	C
ATOM	287	C	ARG	A	20	70.231	9.491	39.242	1.00 30.62	C
ATOM	288	O	ARG	A	20	70.957	9.091	40.129	1.00 33.00	O
ATOM	289	CB	ARG	A	20	71.201	9.743	36.957	1.00 11.71	C
ATOM	290	CG	ARG	A	20	72.610	9.781	37.449	1.00 23.79	C
ATOM	291	CD	ARG	A	20	72.881	11.107	38.060	1.00 36.76	C
ATOM	292	NE	ARG	A	20	74.297	11.443	38.062	1.00 48.34	N
ATOM	293	CZ	ARG	A	20	74.990	11.841	36.988	1.00100.00	C
ATOM	294	NH1	ARG	A	20	74.393	11.931	35.808	1.00100.00	N
ATOM	295	NH2	ARG	A	20	76.289	12.139	37.076	1.00100.00	N
ATOM	296	N	ARG	A	21	69.368	10.461	39.439	1.00 22.10	N
ATOM	297	CA	ARG	A	21	69.216	11.052	40.750	1.00 17.45	C
ATOM	298	C	ARG	A	21	68.721	10.007	41.730	1.00 26.71	C
ATOM	299	O	ARG	A	21	69.147	10.001	42.885	1.00 30.27	O
ATOM	300	CB	ARG	A	21	68.142	12.144	40.708	1.00 17.93	C
ATOM	301	CG	ARG	A	21	68.682	13.522	40.321	1.00 27.57	C
ATOM	302	CD	ARG	A	21	67.586	14.599	40.130	1.00 23.02	C
ATOM	303	NE	ARG	A	21	67.619	15.000	38.743	1.00 55.12	N
ATOM	304	CZ	ARG	A	21	66.538	15.103	37.995	1.00 10.55	C
ATOM	305	NH1	ARG	A	21	65.343	14.974	38.552	1.00 29.80	N
ATOM	306	NH2	ARG	A	21	66.665	15.435	36.715	1.00 61.45	N
ATOM	307	N	GLN	A	22	67.713	9.223	41.345	1.00 27.48	N
ATOM	308	CA	GLN	A	22	67.167	8.257	42.313	1.00 24.79	C
ATOM	309	C	GLN	A	22	68.137	7.127	42.547	1.00 31.37	C
ATOM	310	O	GLN	A	22	68.394	6.724	43.685	1.00 27.47	O
ATOM	311	CB	GLN	A	22	65.818	7.706	41.894	1.00 17.11	C
ATOM	312	CG	GLN	A	22	64.921	8.745	41.243	1.00 66.14	C
ATOM	313	CD	GLN	A	22	63.425	8.456	41.397	1.00 41.27	C
ATOM	314	OE1	GLN	A	22	63.002	7.329	41.762	1.00 29.34	O
ATOM	315	NE2	GLN	A	22	62.610	9.464	41.046	1.00 20.12	N
ATOM	316	N	LEU	A	23	68.697	6.652	41.448	1.00 27.99	N
ATOM	317	CA	LEU	A	23	69.649	5.575	41.500	1.00 24.48	C
ATOM	318	C	LEU	A	23	70.828	5.971	42.334	1.00 28.87	C
ATOM	319	O	LEU	A	23	71.288	5.218	43.165	1.00 30.79	O
ATOM	320	CB	LEU	A	23	70.036	5.107	40.089	1.00 22.72	C
ATOM	321	CG	LEU	A	23	68.966	4.072	39.658	1.00 26.16	C
ATOM	322	CD1	LEU	A	23	69.271	3.083	38.481	1.00 24.80	C
ATOM	323	CD2	LEU	A	23	68.427	3.284	40.835	1.00 22.91	C
ATOM	324	N	GLU	A	24	71.279	7.192	42.153	1.00 28.77	N

ATOM	325	CA	GLU	A	24	72.419	7.675	42.909	1.00 33.79	C
ATOM	326	C	GLU	A	24	72.363	7.388	44.412	1.00 35.94	C
ATOM	327	O	GLU	A	24	73.381	7.140	45.031	1.00 39.07	O
ATOM	328	CB	GLU	A	24	72.647	9.165	42.653	1.00 36.21	C
ATOM	329	CG	GLU	A	24	74.068	9.482	42.243	1.00 42.54	C
ATOM	330	CD	GLU	A	24	74.158	10.689	41.333	1.00 89.51	C
ATOM	331	OE1	GLU	A	24	73.386	11.663	41.549	1.00 43.21	O
ATOM	332	OE2	GLU	A	24	74.994	10.646	40.398	1.00 66.28	O
ATOM	333	N	GLN	A	25	71.182	7.422	45.000	1.00 45.70	N
ATOM	334	CA	GLN	A	25	71.039	7.152	46.432	1.00 47.57	C
ATOM	335	C	GLN	A	25	70.887	5.669	46.740	1.00 67.34	C
ATOM	336	O	GLN	A	25	70.285	5.286	47.726	1.00 74.06	O
ATOM	337	CB	GLN	A	25	69.783	7.842	46.905	1.00 51.85	C
ATOM	338	CG	GLN	A	25	69.500	9.084	46.109	1.00 44.91	C
ATOM	339	CD	GLN	A	25	68.419	9.913	46.742	1.00100.00	C
ATOM	340	OE1	GLN	A	25	68.271	9.947	47.972	1.00100.00	O
ATOM	341	NE2	GLN	A	25	67.624	10.602	45.911	1.00100.00	N
ATOM	342	N	ARG	A	26	71.322	4.831	45.825	1.00 75.37	N
ATOM	343	CA	ARG	A	26	71.182	3.407	46.026	1.00 74.87	C
ATOM	344	C	ARG	A	26	72.568	2.791	46.147	1.00 74.08	C
ATOM	345	O	ARG	A	26	73.440	2.997	45.289	1.00 77.00	O
ATOM	346	CB	ARG	A	26	70.390	2.790	44.885	1.00 52.44	C
ATOM	347	CG	ARG	A	26	68.916	2.927	45.070	1.00 43.51	C
ATOM	348	CD	ARG	A	26	68.428	1.752	45.864	1.00 40.70	C
ATOM	349	NE	ARG	A	26	67.200	1.176	45.338	1.00 42.33	N
ATOM	350	CZ	ARG	A	26	67.126	0.508	44.196	1.00 32.07	C
ATOM	351	NH1	ARG	A	26	68.215	0.324	43.486	1.00 44.02	N
ATOM	352	NH2	ARG	A	26	65.968	0.017	43.771	1.00 77.32	N
ATOM	353	N	GLY	A	27	72.778	2.114	47.266	1.00 46.30	N
ATOM	354	CA	GLY	A	27	74.060	1.531	47.549	1.00 46.82	C
ATOM	355	C	GLY	A	27	74.140	0.165	46.923	1.00 55.45	C
ATOM	356	O	GLY	A	27	75.204	-0.453	46.877	1.00 64.43	O
ATOM	357	N	ASP	A	28	73.017	-0.315	46.428	1.00 40.98	N
ATOM	358	CA	ASP	A	28	73.016	-1.647	45.861	1.00 40.35	C
ATOM	359	C	ASP	A	28	73.266	-1.536	44.400	1.00 39.55	C
ATOM	360	O	ASP	A	28	73.109	-2.518	43.654	1.00 48.80	O
ATOM	361	CB	ASP	A	28	71.680	-2.335	46.127	1.00 47.80	C
ATOM	362	CG	ASP	A	28	70.503	-1.373	46.064	1.00 35.34	C
ATOM	363	OD1	ASP	A	28	70.705	-0.140	46.095	1.00 39.23	O
ATOM	364	OD2	ASP	A	28	69.383	-1.870	45.872	1.00 69.86	O
ATOM	365	N	VAL	A	29	73.651	-0.329	43.996	1.00 31.03	N
ATOM	366	CA	VAL	A	29	73.881	-0.050	42.591	1.00 28.44	C
ATOM	367	C	VAL	A	29	75.166	-0.676	42.281	1.00 28.00	C
ATOM	368	O	VAL	A	29	75.505	1.699	42.892	1.00 34.83	O
ATOM	369	CB	VAL	A	29	72.696	0.760	42.000	1.00 30.68	C

ATOM	370	CG1	VAL	A	29	72.935	1.088	40.549	1.00	23.65	C
ATOM	371	CG2	VAL	A	29	71.416	-0.028	42.156	1.00	27.95	C
ATOM	372	N	GLU	A	30	75.824	0.219	41.230	1.00	30.76	N
ATOM	373	CA	GLU	A	30	76.995	0.924	40.736	1.00	28.38	C
ATOM	374	C	GLU	A	30	76.678	1.471	39.332	1.00	31.03	C
ATOM	375	O	GLU	A	30	76.368	0.720	38.397	1.00	26.64	O
ATOM	376	CB	GLU	A	30	78.199	0.006	40.722	1.00	31.84	C
ATOM	377	CG	GLU	A	30	79.355	0.539	41.533	1.00	89.26	C
ATOM	378	CD	GLU	A	30	80.667	0.264	40.858	1.00	100.00	C
ATOM	379	OE1	GLU	A	30	81.082	-0.922	40.872	1.00	88.94	O
ATOM	380	OE2	GLU	A	30	81.202	1.206	40.219	1.00	100.00	O
ATOM	381	N	LEU	A	31	76.665	2.789	39.207	1.00	22.24	N
ATOM	382	CA	LEU	A	31	76.269	3.391	37.945	1.00	29.37	C
ATOM	383	C	LEU	A	31	77.404	3.507	36.941	1.00	25.79	C
ATOM	384	O	LEU	A	31	78.485	3.969	37.256	1.00	29.41	O
ATOM	385	CB	LEU	A	31	75.632	4.760	38.191	1.00	30.20	C
ATOM	386	CG	LEU	A	31	74.329	4.763	38.994	1.00	29.37	C
ATOM	387	CD1	LEU	A	31	73.841	6.143	39.240	1.00	23.43	C
ATOM	388	CD2	LEU	A	31	73.275	3.962	38.281	1.00	23.04	C
ATOM	389	N	VAL	A	32	77.146	3.100	35.711	1.00	21.94	N
ATOM	390	CA	VAL	A	32	78.143	3.265	34.685	1.00	25.48	C
ATOM	391	C	VAL	A	32	77.535	4.242	33.669	1.00	38.76	C
ATOM	392	O	VAL	A	32	76.429	3.999	33.180	1.00	29.70	O
ATOM	393	CB	VAL	A	32	78.517	1.902	34.055	1.00	34.25	C
ATOM	394	CG1	VAL	A	32	79.587	2.079	32.970	1.00	30.56	C
ATOM	395	CG2	VAL	A	32	79.003	0.950	35.139	1.00	25.27	C
ATOM	396	N	LEU	A	33	78.219	5.375	33.457	1.00	30.19	N
ATOM	397	CA	LEU	A	33	77.732	6.463	32.621	1.00	22.71	C
ATOM	398	C	LEU	A	33	78.727	6.979	31.645	1.00	29.55	C
ATOM	399	O	LEU	A	33	79.896	7.152	31.988	1.00	30.09	O
ATOM	400	CB	LEU	A	33	77.423	7.635	33.514	1.00	19.75	C
ATOM	401	CG	LEU	A	33	76.729	7.200	34.779	1.00	19.38	C
ATOM	402	CD1	LEU	A	33	76.814	8.344	35.762	1.00	27.24	C
ATOM	403	CD2	LEU	A	33	75.271	6.913	34.444	1.00	22.07	C
ATOM	404	N	ARG	A	34	78.239	7.421	30.496	1.00	15.09	N
ATOM	405	CA	ARG	A	34	79.154	8.008	29.541	1.00	26.04	C
ATOM	406	C	ARG	A	34	78.469	9.173	28.916	1.00	36.57	C
ATOM	407	O	ARG	A	34	77.288	9.130	28.651	1.00	38.59	O
ATOM	408	CB	ARG	A	34	79.486	7.048	28.398	1.00	22.89	C
ATOM	409	CG	ARG	A	34	80.579	6.081	28.706	1.00	23.29	C
ATOM	410	CD	ARG	A	34	81.370	6.575	29.860	1.00	52.06	C
ATOM	411	NE	ARG	A	34	81.783	5.458	30.711	1.00	80.25	N
ATOM	412	CZ	ARG	A	34	82.646	4.530	30.323	1.00	41.94	C
ATOM	413	NH1	ARG	A	34	83.173	4.596	29.104	1.00	53.02	N
ATOM	414	NH2	ARG	A	34	82.983	3.547	31.148	1.00	25.56	N

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ATOM    415  N   THR A  35      79.248  10.156  28.539  1.00 31.58           N
ATOM    416  CA  THR A  35      78.703  11.282  27.833  1.00 29.33           C
ATOM    417  C   THR A  35      78.719  10.951  26.340  1.00 32.53           C
ATOM    418  O   THR A  35      79.350   9.944  25.962  1.00 28.08           O
ATOM    419  CB  THR A  35      79.527  12.527  28.145  1.00 37.49           C
ATOM    420  OG1 THR A  35      80.844  12.429  27.560  1.00 31.91           O
ATOM    421  CG2 THR A  35      79.627  12.642  29.651  1.00 19.38           C
ATOM    422  N   ARG A  36      78.032  11.780  25.529  1.00 30.02           N
ATOM    423  CA  ARG A  36      78.002  11.639  24.056  1.00 29.37           C
ATOM    424  C   ARG A  36      79.406  11.765  23.503  1.00 31.46           C
ATOM    425  O   ARG A  36      79.772  11.012  22.591  1.00 36.56           O
ATOM    426  CB  ARG A  36      77.054  12.650  23.354  1.00 37.34           C
ATOM    427  CG  ARG A  36      76.937  12.465  21.846-99.00 49.47           C
ATOM    428  CD  ARG A  36      76.020  13.515  21.232-99.00 63.09           C
ATOM    429  NE  ARG A  36      75.528  13.124  19.915-99.00 75.23           N
ATOM    430  CZ  ARG A  36      74.381  13.549  19.391-99.00 91.44           C
ATOM    431  NH1 ARG A  36      73.605  14.375  20.079-99.00 79.32           N
ATOM    432  NH2 ARG A  36      74.009  13.144  18.185-99.00 78.73           N
ATOM    433  N   ASP A  37      80.217  12.677  24.063  1.00 41.30           N
ATOM    434  CA  ASP A  37      81.606  12.710  23.601  1.00 44.91           C
ATOM    435  C   ASP A  37      82.410  11.481  24.043  1.00 24.99           C
ATOM    436  O   ASP A  37      83.211  10.978  23.261  1.00 42.22           O
ATOM    437  CB  ASP A  37      82.347  14.048  23.718-99.00 47.07           C
ATOM    438  CG  ASP A  37      81.881  14.887  24.876-99.00 62.99           C
ATOM    439  OD1 ASP A  37      80.679  14.839  25.204-99.00 64.45           O
ATOM    440  OD2 ASP A  37      82.711  15.638  25.429-99.00 69.84           O
ATOM    441  N   GLU A  38      82.129  10.950  25.235  1.00 19.39           N
ATOM    442  CA  GLU A  38      82.790   9.717  25.682  1.00 27.84           C
ATOM    443  C   GLU A  38      82.203   8.527  24.901  1.00 37.14           C
ATOM    444  O   GLU A  38      82.873   7.511  24.699  1.00 35.04           O
ATOM    445  CB  GLU A  38      82.691   9.435  27.207  1.00 25.18           C
ATOM    446  CG  GLU A  38      83.116  10.549  28.183  1.00 37.45           C
ATOM    447  CD  GLU A  38      82.807  10.212  29.655  1.00 21.13           C
ATOM    448  OE1 GLU A  38      81.623   9.997  30.014  1.00 55.97           O
ATOM    449  OE2 GLU A  38      83.757   9.978  30.419  1.00 98.78           O
ATOM    450  N   LEU A  39      80.948   8.610  24.478  1.00 25.52           N
ATOM    451  CA  LEU A  39      80.440   7.483  23.739  1.00 18.17           C
ATOM    452  C   LEU A  39      79.291   7.764  22.825  1.00 20.34           C
ATOM    453  O   LEU A  39      78.152   7.810  23.259  1.00 26.35           O
ATOM    454  CB  LEU A  39      80.123   6.313  24.657  1.00 14.56           C
ATOM    455  CG  LEU A  39      79.410   5.075  24.058  1.00 19.52           C
ATOM    456  CD1 LEU A  39      80.205   4.392  22.994  1.00 18.84           C
ATOM    457  CD2 LEU A  39      78.890   4.051  25.084  1.00 17.41           C
ATOM    458  N   ASN A  40      79.598   7.880  21.543  1.00 16.73           N
ATOM    459  CA  ASN A  40      78.548   7.971  20.540  1.00 21.55           C
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ATOM    460  C   ASN A  40      77.798   6.649  20.308  1.00 24.53           C
ATOM    461  O   ASN A  40      78.328   5.720  19.688  1.00 19.96           O
ATOM    462  CB  ASN A  40      79.130   8.367  19.216  1.00 18.45           C
ATOM    463  CG  ASN A  40      78.054   8.727  18.225  1.00 42.19           C
ATOM    464  OD1 ASN A  40      78.327   9.093  17.080  1.00 38.89           O
ATOM    465  ND2 ASN A  40      76.827   8.730  18.697  1.00 23.71           N
ATOM    466  N   LEU A  41      76.543   6.622  20.754  1.00 21.08           N
ATOM    467  CA  LEU A  41      75.649   5.465  20.650  1.00 15.03           C
ATOM    468  C   LEU A  41      75.225   5.068  19.213  1.00 18.22           C
ATOM    469  O   LEU A  41      74.681   3.971  18.980  1.00 15.72           O
ATOM    470  CB  LEU A  41      74.426   5.705  21.532  1.00 15.85           C
ATOM    471  CG  LEU A  41      74.822   6.029  22.974  1.00 21.90           C
ATOM    472  CD1 LEU A  41      73.604   6.413  23.749  1.00 20.59           C
ATOM    473  CD2 LEU A  41      75.481   4.796  23.609  1.00 17.97           C
ATOM    474  N   LEU A  42      75.542   5.916  18.238  1.00 12.45           N
ATOM    475  CA  LEU A  42      75.256   5.607  16.831  1.00 15.99           C
ATOM    476  C   LEU A  42      76.290   4.680  16.280  1.00 26.18           C
ATOM    477  O   LEU A  42      76.066   4.039  15.257  1.00 22.41           O
ATOM    478  CB  LEU A  42      75.282   6.873  15.984  1.00 17.85           C
ATOM    479  CG  LEU A  42      74.180   7.854  16.399  1.00 30.70           C
ATOM    480  CD1 LEU A  42      74.318   9.184  15.704  1.00 24.31           C
ATOM    481  CD2 LEU A  42      72.764   7.241  16.208  1.00 31.13           C
ATOM    482  N   ASP A  43      77.462   4.705  16.911  1.00 26.87           N
ATOM    483  CA  ASP A  43      78.579   3.875  16.486  1.00 19.29           C
ATOM    484  C   ASP A  43      78.583   2.519  17.163  1.00 13.33           C
ATOM    485  O   ASP A  43      79.051   2.348  18.297  1.00 18.75           O
ATOM    486  CB  ASP A  43      79.870   4.580  16.776  1.00 31.06           C
ATOM    487  CG  ASP A  43      81.083   3.758  16.380  1.00 30.68           C
ATOM    488  OD1 ASP A  43      80.971   2.551  16.082  1.00 32.36           O
ATOM    489  OD2 ASP A  43      82.187   4.308  16.499  1.00 37.83           O
ATOM    490  N   SER A  44      78.139   1.544  16.377  1.00 16.89           N
ATOM    491  CA  SER A  44      77.978   0.173  16.789  1.00 17.67           C
ATOM    492  C   SER A  44      79.237  -0.463  17.392  1.00 20.40           C
ATOM    493  O   SER A  44      79.206  -1.126  18.444  1.00 26.27           O
ATOM    494  CB  SER A  44      77.504  -0.617  15.581  1.00 13.85           C
ATOM    495  OG  SER A  44      76.800  -1.740  16.063  1.00 43.83           O
ATOM    496  N   ARG A  45      80.335  -0.301  16.682  1.00 15.63           N
ATOM    497  CA  ARG A  45      81.616  -0.788  17.154  1.00 19.94           C
ATOM    498  C   ARG A  45      81.910  -0.225  18.521  1.00 29.48           C
ATOM    499  O   ARG A  45      82.244  -0.937  19.457  1.00 27.65           O
ATOM    500  CB  ARG A  45      82.684  -0.261  16.203  1.00 27.46           C
ATOM    501  CG  ARG A  45      83.463  -1.338  15.495  1.00 92.03           C
ATOM    502  CD  ARG A  45      84.854  -1.418  16.077  1.00100.00           C
ATOM    503  NE  ARG A  45      85.636  -2.533  15.527  1.00100.00           N
ATOM    504  CZ  ARG A  45      86.092  -3.570  16.236  1.00100.00           C
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ATOM    505  NH1 ARG A  45      85.791  -3.695  17.547  1.00100.00           N
ATOM    506  NH2 ARG A  45      86.773  -4.544  15.642  1.00100.00           N
ATOM    507  N   ALA A  46      81.772   1.090  18.629  1.00 31.04           N
ATOM    508  CA  ALA A  46      82.045   1.743  19.881  1.00 24.72           C
ATOM    509  C   ALA A  46      81.111   1.176  20.899  1.00 17.73           C
ATOM    510  O   ALA A  46      81.512   0.825  22.027  1.00 22.73           O
ATOM    511  CB  ALA A  46      81.839   3.221  19.751  1.00 27.16           C
ATOM    512  N   VAL A  47      79.835   1.119  20.531  1.00 17.54           N
ATOM    513  CA  VAL A  47      78.878   0.608  21.508  1.00 21.41           C
ATOM    514  C   VAL A  47      79.262  -0.812  21.914  1.00 30.25           C
ATOM    515  O   VAL A  47      79.192  -1.202  23.097  1.00 15.85           O
ATOM    516  CB  VAL A  47      77.470   0.668  20.989  1.00 18.59           C
ATOM    517  CG1 VAL A  47      76.503   0.042  22.012  1.00 16.88           C
ATOM    518  CG2 VAL A  47      77.115   2.096  20.756  1.00 16.28           C
ATOM    519  N   HIS A  48      79.692  -1.585  20.920  1.00 21.00           N
ATOM    520  CA  HIS A  48      80.028  -2.969  21.192  1.00 20.17           C
ATOM    521  C   HIS A  48      81.268  -3.079  22.117  1.00 32.98           C
ATOM    522  O   HIS A  48      81.289  -3.850  23.102  1.00 28.20           O
ATOM    523  CB  HIS A  48      80.063  -3.801  19.855  1.00 14.93           C
ATOM    524  CG  HIS A  48      78.686  -4.172  19.338  1.00 26.67           C
ATOM    525  ND1 HIS A  48      78.085  -5.394  19.600  1.00 28.83           N
ATOM    526  CD2 HIS A  48      77.758  -3.448  18.659  1.00 25.56           C
ATOM    527  CE1 HIS A  48      76.887  -5.430  19.043  1.00 20.08           C
ATOM    528  NE2 HIS A  48      76.660  -4.260  18.475  1.00 25.22           N
ATOM    529  N   ASP A  49      82.217  -2.170  21.902  1.00 22.62           N
ATOM    530  CA  ASP A  49      83.455  -2.169  22.674  1.00 24.23           C
ATOM    531  C   ASP A  49      83.171  -1.899  24.122  1.00 38.72           C
ATOM    532  O   ASP A  49      83.708  -2.551  25.027  1.00 35.44           O
ATOM    533  CB  ASP A  49      84.396  -1.112  22.127  1.00 30.29           C
ATOM    534  CG  ASP A  49      84.991  -1.503  20.775  1.00 52.45           C
ATOM    535  OD1 ASP A  49      85.007  -2.726  20.449  1.00 42.67           O
ATOM    536  OD2 ASP A  49      85.416  -0.587  20.029  1.00 73.76           O
ATOM    537  N   PHE A  50      82.294  -0.929  24.324  1.00 32.19           N
ATOM    538  CA  PHE A  50      81.902  -0.550  25.649  1.00 29.76           C
ATOM    539  C   PHE A  50      81.299  -1.765  26.359  1.00 30.31           C
ATOM    540  O   PHE A  50      81.715  -2.124  27.449  1.00 29.22           O
ATOM    541  CB  PHE A  50      80.892   0.610  25.576  1.00 23.82           C
ATOM    542  CG  PHE A  50      80.137   0.843  26.859  1.00 19.13           C
ATOM    543  CD1 PHE A  50      80.740   1.515  27.931  1.00 20.14           C
ATOM    544  CD2 PHE A  50      78.835   0.360  27.018  1.00 13.99           C
ATOM    545  CE1 PHE A  50      80.034   1.742  29.129  1.00 25.81           C
ATOM    546  CE2 PHE A  50      78.114   0.553  28.212  1.00 22.84           C
ATOM    547  CZ  PHE A  50      78.698   1.276  29.259  1.00 23.40           C
ATOM    548  N   PHE A  51      80.280  -2.367  25.768  1.00 21.75           N
ATOM    549  CA  PHE A  51      79.655  -3.451  26.457  1.00 22.61           C
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ATOM 550 C PHE A 51 80.646 -4.603 26.612 1.00 34.01 C
ATOM 551 O PHE A 51 80.550 -5.401 27.590 1.00 25.28 O
ATOM 552 CB PHE A 51 78.389 -3.898 25.751 1.00 22.63 C
ATOM 553 CG PHE A 51 77.158 -3.140 26.170 1.00 27.58 C
ATOM 554 CD1 PHE A 51 76.426 -3.525 27.280 1.00 21.78 C
ATOM 555 CD2 PHE A 51 76.663 -2.100 25.380 1.00 19.55 C
ATOM 556 CE1 PHE A 51 75.267 -2.796 27.662 1.00 28.34 C
ATOM 557 CE2 PHE A 51 75.492 -1.403 25.734 1.00 14.47 C
ATOM 558 CZ PHE A 51 74.797 -1.744 26.878 1.00 14.55 C
ATOM 559 N ALA A 52 81.576 -4.706 25.659 1.00 26.43 N
ATOM 560 CA ALA A 52 82.587 -5.793 25.714 1.00 29.44 C
ATOM 561 C ALA A 52 83.687 -5.560 26.768 1.00 43.76 C
ATOM 562 O ALA A 52 84.502 -6.446 27.022 1.00 40.33 O
ATOM 563 CB ALA A 52 83.228 -6.049 24.344 1.00 24.25 C
ATOM 564 N SER A 53 83.702 -4.382 27.385 1.00 31.96 N
ATOM 565 CA SER A 53 84.705 -4.090 28.377 1.00 21.06 C
ATOM 566 C SER A 53 84.196 -3.625 29.709 1.00 26.41 C
ATOM 567 O SER A 53 84.985 -3.492 30.611 1.00 36.12 O
ATOM 568 CB SER A 53 85.709 -3.088 27.843 1.00 14.22 C
ATOM 569 OG SER A 53 85.140 -1.807 27.790 1.00 56.90 O
ATOM 570 N GLU A 54 82.892 -3.431 29.874 1.00 22.38 N
ATOM 571 CA GLU A 54 82.380 -2.893 31.139 1.00 17.27 C
ATOM 572 C GLU A 54 81.584 -3.735 32.118 1.00 26.32 C
ATOM 573 O GLU A 54 81.229 -3.281 33.191 1.00 37.43 O
ATOM 574 CB GLU A 54 81.677 -1.563 30.906 1.00 27.30 C
ATOM 575 CG GLU A 54 82.573 -0.543 30.262 1.00 44.77 C
ATOM 576 CD GLU A 54 83.669 -0.142 31.194 1.00 86.31 C
ATOM 577 OE1 GLU A 54 83.392 -0.232 32.428 1.00 50.11 O
ATOM 578 OE2 GLU A 54 84.785 0.198 30.692 1.00 50.99 O
ATOM 579 N ARG A 55 81.268 -4.971 31.804 1.00 29.63 N
ATOM 580 CA ARG A 55 80.636 -5.748 32.854 1.00 33.32 C
ATOM 581 C ARG A 55 79.347 -5.149 33.378 1.00 38.45 C
ATOM 582 O ARG A 55 79.214 -4.897 34.576 1.00 40.18 O
ATOM 583 CB ARG A 55 81.621 -5.875 34.045 1.00 57.61 C
ATOM 584 CG ARG A 55 82.666 -7.028 33.960 1.00100.00 C
ATOM 585 CD ARG A 55 82.805 -7.805 35.305 1.00100.00 C
ATOM 586 NE ARG A 55 82.838 -9.270 35.146 1.00100.00 N
ATOM 587 CZ ARG A 55 83.206 -10.129 36.102 1.00100.00 C
ATOM 588 NH1 ARG A 55 83.583 -9.681 37.301 1.00100.00 N
ATOM 589 NH2 ARG A 55 83.208 -11.440 35.855 1.00100.00 N
ATOM 590 N ILE A 56 78.367 -5.029 32.491 1.00 42.25 N
ATOM 591 CA ILE A 56 77.064 -4.434 32.794 1.00 25.49 C
ATOM 592 C ILE A 56 75.982 -5.474 33.244 1.00 20.18 C
ATOM 593 O ILE A 56 75.897 -6.579 32.704 1.00 24.74 O
ATOM 594 CB ILE A 56 76.672 -3.512 31.531 1.00 26.89 C

ATOM	595	CG1	ILE	A	56	77.643	-2.301	31.442	1.00 18.30	C
ATOM	596	CG2	ILE	A	56	75.214	-3.016	31.549	1.00 19.84	C
ATOM	597	CD1	ILE	A	56	77.998	-1.936	30.026	1.00 60.42	C
ATOM	598	N	ASP	A	57	75.166	-5.133	34.237	1.00 16.84	N
ATOM	599	CA	ASP	A	57	74.040	-5.999	34.630	1.00 16.33	C
ATOM	600	C	ASP	A	57	72.676	-5.451	34.123	1.00 28.40	C
ATOM	601	O	ASP	A	57	71.836	-6.198	33.657	1.00 25.50	O
ATOM	602	CB	ASP	A	57	74.009	-6.194	36.164	1.00 16.94	C
ATOM	603	CG	ASP	A	57	75.369	-6.720	36.703	1.00 34.27	C
ATOM	604	OD1	ASP	A	57	75.875	-7.729	36.141	1.00 31.76	O
ATOM	605	OD2	ASP	A	57	76.040	-6.007	37.499	1.00 28.36	O
ATOM	606	N	GLN	A	58	72.443	-4.152	34.220	1.00 28.91	N
ATOM	607	CA	GLN	A	58	71.183	-3.590	33.755	1.00 25.68	C
ATOM	608	C	GLN	A	58	71.425	-2.364	32.881	1.00 23.21	C
ATOM	609	O	GLN	A	58	72.403	-1.620	33.067	1.00 18.16	O
ATOM	610	CB	GLN	A	58	70.342	-3.151	34.946	1.00 33.14	C
ATOM	611	CG	GLN	A	58	69.798	-4.241	35.807	1.00 30.00	C
ATOM	612	CD	GLN	A	58	69.226	-3.712	37.105	1.00 27.18	C
ATOM	613	OE1	GLN	A	58	68.722	-2.601	37.161	1.00 31.20	O
ATOM	614	NE2	GLN	A	58	69.455	-4.436	38.186	1.00 16.89	N
ATOM	615	N	VAL	A	59	70.496	-2.138	31.961	1.00 18.35	N
ATOM	616	CA	VAL	A	59	70.562	-0.998	31.045	1.00 15.59	C
ATOM	617	C	VAL	A	59	69.238	-0.240	31.039	1.00 26.28	C
ATOM	618	O	VAL	A	59	68.178	-0.820	30.762	1.00 19.51	O
ATOM	619	CB	VAL	A	59	70.707	-1.456	29.601	1.00 15.32	C
ATOM	620	CG1	VAL	A	59	70.477	-0.274	28.649	1.00 11.93	C
ATOM	621	CG2	VAL	A	59	72.080	-2.111	29.364	1.00 15.83	C
ATOM	622	N	TYR	A	60	69.306	1.064	31.293	1.00 21.71	N
ATOM	623	CA	TYR	A	60	68.113	1.927	31.197	1.00 21.40	C
ATOM	624	C	TYR	A	60	68.289	2.756	29.928	1.00 18.69	C
ATOM	625	O	TYR	A	60	69.250	3.532	29.796	1.00 15.51	O
ATOM	626	CB	TYR	A	60	68.021	2.817	32.413	1.00 17.24	C
ATOM	627	CG	TYR	A	60	67.493	2.131	33.658	1.00 19.71	C
ATOM	628	CD1	TYR	A	60	68.345	1.583	34.586	1.00 21.14	C
ATOM	629	CD2	TYR	A	60	66.154	2.223	33.991	1.00 20.16	C
ATOM	630	CE1	TYR	A	60	67.835	1.080	35.794	1.00 19.11	C
ATOM	631	CE2	TYR	A	60	65.648	1.698	35.163	1.00 10.77	C
ATOM	632	CZ	TYR	A	60	66.476	1.094	36.054	1.00 20.07	C
ATOM	633	OH	TYR	A	60	65.921	0.585	37.248	1.00 16.04	O
ATOM	634	N	LEU	A	61	67.491	2.452	28.916	1.00 17.46	N
ATOM	635	CA	LEU	A	61	67.685	3.053	27.585	1.00 20.17	C
ATOM	636	C	LEU	A	61	67.003	4.412	27.409	1.00 23.36	C
ATOM	637	O	LEU	A	61	65.925	4.526	26.799	1.00 14.86	O
ATOM	638	CB	LEU	A	61	67.267	2.060	26.485	1.00 14.78	C
ATOM	639	CG	LEU	A	61	68.117	2.142	25.208	1.00 15.52	C

ATOM	640	CD1	LEU	A	61	67.815	1.010	24.109	1.00	7.75	C
ATOM	641	CD2	LEU	A	61	68.087	3.541	24.580	1.00	15.20	C
ATOM	642	N	ALA	A	62	67.656	5.434	27.956	1.00	20.35	N
ATOM	643	CA	ALA	A	62	67.120	6.784	27.963	1.00	18.55	C
ATOM	644	C	ALA	A	62	67.779	7.739	26.949	1.00	18.57	C
ATOM	645	O	ALA	A	62	67.455	8.924	26.920	1.00	24.31	O
ATOM	646	CB	ALA	A	62	67.071	7.377	29.439	1.00	11.69	C
ATOM	647	N	ALA	A	63	68.681	7.231	26.101	1.00	14.09	N
ATOM	648	CA	ALA	A	63	69.249	8.095	25.052	1.00	12.84	C
ATOM	649	C	ALA	A	63	68.310	8.005	23.877	1.00	27.00	C
ATOM	650	O	ALA	A	63	67.845	6.916	23.511	1.00	24.51	O
ATOM	651	CB	ALA	A	63	70.665	7.660	24.634	1.00	4.89	C
ATOM	652	N	ALA	A	64	68.076	9.148	23.262	1.00	21.05	N
ATOM	653	CA	ALA	A	64	67.202	9.286	22.086	1.00	13.50	C
ATOM	654	C	ALA	A	64	67.435	10.664	21.416	1.00	28.08	C
ATOM	655	O	ALA	A	64	67.987	11.600	22.021	1.00	26.63	O
ATOM	656	CB	ALA	A	64	65.642	9.171	22.518	1.00	7.63	C
ATOM	657	N	LYS	A	65	66.953	10.781	20.182	1.00	23.98	N
ATOM	658	CA	LYS	A	65	66.966	12.012	19.409	1.00	20.47	C
ATOM	659	C	LYS	A	65	65.488	12.443	19.551	1.00	24.37	C
ATOM	660	O	LYS	A	65	64.594	11.807	18.976	1.00	20.29	O
ATOM	661	CB	LYS	A	65	67.317	11.658	17.951	1.00	25.59	C
ATOM	662	CG	LYS	A	65	66.808	12.630	16.923	1.00	27.54	C
ATOM	663	CD	LYS	A	65	67.518	13.926	17.169	1.00	21.08	C
ATOM	664	CE	LYS	A	65	67.316	14.905	16.029	1.00	55.15	C
ATOM	665	NZ	LYS	A	65	67.876	16.263	16.392	1.00	81.63	N
ATOM	666	N	VAL	A	66	65.228	13.362	20.485	1.00	22.47	N
ATOM	667	CA	VAL	A	66	63.873	13.850	20.755	1.00	18.99	C
ATOM	668	C	VAL	A	66	63.711	15.343	20.394	1.00	31.44	C
ATOM	669	O	VAL	A	66	64.665	16.107	20.460	1.00	34.61	O
ATOM	670	CB	VAL	A	66	63.440	13.623	22.204	1.00	16.66	C
ATOM	671	CG1	VAL	A	66	64.269	12.623	22.869	1.00	15.01	C
ATOM	672	CG2	VAL	A	66	63.379	14.904	22.950	1.00	19.21	C
ATOM	673	N	GLY	A	67	62.514	15.755	19.994	1.00	18.03	N
ATOM	674	CA	GLY	A	67	62.298	17.149	19.614	1.00	14.90	C
ATOM	675	C	GLY	A	67	60.792	17.518	19.585	1.00	32.35	C
ATOM	676	O	GLY	A	67	59.922	16.666	19.888	1.00	18.88	O
ATOM	677	N	GLY	A	68	60.503	18.787	19.256	1.00	23.21	N
ATOM	678	CA	GLY	A	68	59.132	19.288	19.183	1.00	23.83	C
ATOM	679	C	GLY	A	68	58.540	19.137	17.771	1.00	19.31	C
ATOM	680	O	GLY	A	68	59.165	18.550	16.870	1.00	30.64	O
ATOM	681	N	ILE	A	69	57.343	19.684	17.588	1.00	15.20	N
ATOM	682	CA	ILE	A	69	56.595	19.632	16.317	1.00	16.80	C
ATOM	683	C	ILE	A	69	57.387	20.153	15.112	1.00	19.33	C
ATOM	684	O	ILE	A	69	57.425	19.519	14.061	1.00	14.66	O

ATOM 685 CB ILE A 69 55.257 20.432 16.480 1.00 30.11 C
ATOM 686 CG1 ILE A 69 54.271 19.683 17.385 1.00 24.27 C
ATOM 687 CG2 ILE A 69 54.610 20.749 15.181 1.00 47.53 C
ATOM 688 CD1 ILE A 69 53.259 20.608 18.056 1.00 85.71 C
ATOM 689 N VAL A 70 58.010 21.327 15.269 1.00 23.03 N
ATOM 690 CA VAL A 70 58.797 21.913 14.183 1.00 19.34 C
ATOM 691 C VAL A 70 59.983 21.011 13.840 1.00 24.42 C
ATOM 692 O VAL A 70 60.335 20.829 12.662 1.00 24.14 O
ATOM 693 CB VAL A 70 59.304 23.404 14.467 1.00 21.37 C
ATOM 694 CG1 VAL A 70 60.137 23.907 13.281 1.00 17.79 C
ATOM 695 CG2 VAL A 70 58.136 24.410 14.678 1.00 15.74 C
ATOM 696 N ALA A 71 60.621 20.450 14.861 1.00 19.68 N
ATOM 697 CA ALA A 71 61.782 19.617 14.572 1.00 16.57 C
ATOM 698 C ALA A 71 61.427 18.289 13.910 1.00 23.36 C
ATOM 699 O ALA A 71 61.980 17.923 12.849 1.00 21.84 O
ATOM 700 CB ALA A 71 62.685 19.439 15.805 1.00 9.36 C
ATOM 701 N ASN A 72 60.463 17.598 14.511 1.00 16.80 N
ATOM 702 CA ASN A 72 59.998 16.357 13.923 1.00 18.84 C
ATOM 703 C ASN A 72 59.608 16.539 12.440 1.00 23.87 C
ATOM 704 O ASN A 72 59.919 15.696 11.593 1.00 21.52 O
ATOM 705 CB ASN A 72 58.835 15.806 14.738 1.00 8.60 C
ATOM 706 CG ASN A 72 59.309 15.013 15.911 1.00 23.75 C
ATOM 707 OD1 ASN A 72 59.558 13.809 15.810 1.00 23.98 O
ATOM 708 ND2 ASN A 72 59.572 15.701 16.996 1.00 9.96 N
ATOM 709 N ASN A 73 58.931 17.647 12.138 1.00 23.07 N
ATOM 710 CA ASN A 73 58.521 17.971 10.761 1.00 26.05 C
ATOM 711 C ASN A 73 59.665 18.454 9.817 1.00 26.95 C
ATOM 712 O ASN A 73 59.613 18.276 8.569 1.00 22.13 O
ATOM 713 CB ASN A 73 57.383 19.001 10.800 1.00 14.86 C
ATOM 714 CG ASN A 73 56.015 18.349 10.987 1.00 19.88 C
ATOM 715 OD1 ASN A 73 55.620 17.468 10.217 1.00 27.02 O
ATOM 716 ND2 ASN A 73 55.322 18.732 12.051 1.00 20.78 N
ATOM 717 N THR A 74 60.710 19.029 10.419 1.00 18.69 N
ATOM 718 CA THR A 74 61.845 19.540 9.657 1.00 10.07 C
ATOM 719 C THR A 74 62.968 18.548 9.375 1.00 21.00 C
ATOM 720 O THR A 74 63.537 18.561 8.289 1.00 11.75 O
ATOM 721 CB THR A 74 62.411 20.746 10.306 1.00 29.10 C
ATOM 722 OG1 THR A 74 61.370 21.714 10.457 1.00 23.24 O
ATOM 723 CG2 THR A 74 63.541 21.299 9.452 1.00 21.63 C
ATOM 724 N TYR A 75 63.230 17.636 10.310 1.00 17.10 N
ATOM 725 CA TYR A 75 64.267 16.620 10.112 1.00 9.07 C
ATOM 726 C TYR A 75 63.733 15.203 10.318 1.00 6.17 C
ATOM 727 O TYR A 75 64.143 14.542 11.267 1.00 15.58 O
ATOM 728 CB TYR A 75 65.302 16.825 11.188 1.00 11.89 C
ATOM 729 CG TYR A 75 65.779 18.234 11.252 1.00 27.12 C

ATOM	730	CD1	TYR	A	75	66.712	18.696	10.321	1.00	28.46	C
ATOM	731	CD2	TYR	A	75	65.234	19.151	12.173	1.00	24.83	C
ATOM	732	CE1	TYR	A	75	67.117	20.045	10.305	1.00	28.34	C
ATOM	733	CE2	TYR	A	75	65.652	20.523	12.180	1.00	21.00	C
ATOM	734	CZ	TYR	A	75	66.593	20.940	11.234	1.00	45.42	C
ATOM	735	OH	TYR	A	75	67.066	22.230	11.215	1.00	35.37	O
ATOM	736	N	PRO	A	76	62.759	14.775	9.532	1.00	13.30	N
ATOM	737	CA	PRO	A	76	62.185	13.438	9.742	1.00	14.64	C
ATOM	738	C	PRO	A	76	63.209	12.264	9.618	1.00	14.40	C
ATOM	739	O	PRO	A	76	63.157	11.335	10.409	1.00	20.54	O
ATOM	740	CB	PRO	A	76	61.055	13.366	8.709	1.00	7.83	C
ATOM	741	CG	PRO	A	76	61.447	14.388	7.617	1.00	12.61	C
ATOM	742	CD	PRO	A	76	62.068	15.504	8.455	1.00	11.18	C
ATOM	743	N	ALA	A	77	64.163	12.339	8.681	1.00	15.25	N
ATOM	744	CA	ALA	A	77	65.206	11.312	8.538	1.00	6.79	C
ATOM	745	C	ALA	A	77	66.053	11.166	9.820	1.00	17.22	C
ATOM	746	O	ALA	A	77	66.306	10.069	10.292	1.00	18.74	O
ATOM	747	CB	ALA	A	77	66.097	11.601	7.330	1.00	9.04	C
ATOM	748	N	ASP	A	78	66.466	12.267	10.424	1.00	10.92	N
ATOM	749	CA	ASP	A	78	67.256	12.191	11.659	1.00	11.87	C
ATOM	750	C	ASP	A	78	66.572	11.486	12.827	1.00	16.09	C
ATOM	751	O	ASP	A	78	67.212	10.741	13.601	1.00	18.07	O
ATOM	752	CB	ASP	A	78	67.578	13.609	12.088	1.00	19.16	C
ATOM	753	CG	ASP	A	78	68.424	14.325	11.068	1.00	26.82	C
ATOM	754	OD1	ASP	A	78	68.836	13.694	10.044	1.00	33.93	O
ATOM	755	OD2	ASP	A	78	68.673	15.514	11.316	1.00	32.06	O
ATOM	756	N	PHE	A	79	65.279	11.771	12.975	1.00	14.70	N
ATOM	757	CA	PHE	A	79	64.471	11.192	14.044	1.00	20.69	C
ATOM	758	C	PHE	A	79	64.224	9.707	13.876	1.00	20.22	C
ATOM	759	O	PHE	A	79	64.269	8.987	14.862	1.00	22.37	O
ATOM	760	CB	PHE	A	79	63.144	11.933	14.219	1.00	27.38	C
ATOM	761	CG	PHE	A	79	63.264	13.218	14.990	1.00	28.59	C
ATOM	762	CD1	PHE	A	79	63.137	13.230	16.386	1.00	27.49	C
ATOM	763	CD2	PHE	A	79	63.509	14.415	14.325	1.00	28.20	C
ATOM	764	CE1	PHE	A	79	63.281	14.413	17.109	1.00	21.76	C
ATOM	765	CE2	PHE	A	79	63.625	15.593	15.037	1.00	31.48	C
ATOM	766	CZ	PHE	A	79	63.509	15.582	16.439	1.00	26.31	C
ATOM	767	N	ILE	A	80	63.942	9.249	12.650	1.00	10.79	N
ATOM	768	CA	ILE	A	80	63.828	7.795	12.410	1.00	18.12	C
ATOM	769	C	ILE	A	80	65.197	7.052	12.432	1.00	10.97	C
ATOM	770	O	ILE	A	80	65.406	6.090	13.195	1.00	8.92	O
ATOM	771	CB	ILE	A	80	62.944	7.408	11.148	1.00	17.41	C
ATOM	772	CG1	ILE	A	80	62.651	5.886	11.105	1.00	10.16	C
ATOM	773	CG2	ILE	A	80	63.583	7.888	9.901	1.00	17.46	C
ATOM	774	CD1	ILE	A	80	61.722	5.410	9.980	1.00	7.30	C

ATOM	775	N	TYR A	81	66.151	7.539	11.658	1.00	11.18	N
ATOM	776	CA	TYR A	81	67.488	6.902	11.630	1.00	15.06	C
ATOM	777	C	TYR A	81	68.237	6.782	12.959	1.00	16.83	C
ATOM	778	O	TYR A	81	68.714	5.702	13.383	1.00	16.74	O
ATOM	779	CB	TYR A	81	68.384	7.599	10.616	1.00	9.43	C
ATOM	780	CG	TYR A	81	69.749	6.966	10.541	1.00	22.54	C
ATOM	781	CD1	TYR A	81	69.963	5.824	9.747	1.00	22.37	C
ATOM	782	CD2	TYR A	81	70.818	7.466	11.299	1.00	18.07	C
ATOM	783	CE1	TYR A	81	71.202	5.163	9.746	1.00	15.02	C
ATOM	784	CE2	TYR A	81	72.080	6.893	11.201	1.00	17.37	C
ATOM	785	CZ	TYR A	81	72.255	5.698	10.472	1.00	24.27	C
ATOM	786	OH	TYR A	81	73.491	5.063	10.409	1.00	19.57	O
ATOM	787	N	GLN A	82	68.385	7.918	13.612	1.00	11.39	N
ATOM	788	CA	GLN A	82	69.193	7.930	14.810	1.00	12.23	C
ATOM	789	C	GLN A	82	68.544	7.089	15.834	1.00	14.18	C
ATOM	790	O	GLN A	82	69.180	6.415	16.631	1.00	11.35	O
ATOM	791	CB	GLN A	82	69.280	9.354	15.291	1.00	18.73	C
ATOM	792	CG	GLN A	82	69.986	10.209	14.250	1.00	13.54	C
ATOM	793	CD	GLN A	82	70.285	11.617	14.736	1.00	26.00	C
ATOM	794	OE1	GLN A	82	70.410	11.850	15.927	1.00	22.99	O
ATOM	795	NE2	GLN A	82	70.404	12.561	13.808	1.00	16.59	N
ATOM	796	N	ASN A	83	67.235	7.181	15.869	1.00	11.35	N
ATOM	797	CA	ASN A	83	66.549	6.408	16.860	1.00	13.71	C
ATOM	798	C	ASN A	83	66.623	4.902	16.557	1.00	21.43	C
ATOM	799	O	ASN A	83	66.831	4.101	17.463	1.00	12.10	O
ATOM	800	CB	ASN A	83	65.132	6.945	17.074	1.00	13.51	C
ATOM	801	CG	ASN A	83	65.131	8.245	17.871	1.00	28.91	C
ATOM	802	OD1	ASN A	83	65.628	8.263	18.990	1.00	22.28	O
ATOM	803	ND2	ASN A	83	64.756	9.354	17.237	1.00	20.17	N
ATOM	804	N	MET A	84	66.592	4.517	15.290	1.00	15.63	N
ATOM	805	CA	MET A	84	66.704	3.101	15.007	1.00	15.66	C
ATOM	806	C	MET A	84	68.054	2.588	15.348	1.00	14.66	C
ATOM	807	O	MET A	84	68.148	1.514	15.902	1.00	11.45	O
ATOM	808	CB	MET A	84	66.418	2.815	13.563	1.00	17.59	C
ATOM	809	CG	MET A	84	64.911	2.894	13.220	1.00	14.40	C
ATOM	810	SD	MET A	84	64.638	2.811	11.387	1.00	15.99	S
ATOM	811	CE	MET A	84	65.164	1.105	10.952	1.00	8.90	C
ATOM	812	N	MET A	85	69.098	3.338	15.024	1.00	11.20	N
ATOM	813	CA	MET A	85	70.468	2.879	15.321	1.00	11.67	C
ATOM	814	C	MET A	85	70.779	2.831	16.774	1.00	13.04	C
ATOM	815	O	MET A	85	71.359	1.893	17.265	1.00	15.26	O
ATOM	816	CB	MET A	85	71.525	3.798	14.693	1.00	15.07	C
ATOM	817	CG	MET A	85	71.530	3.726	13.173	1.00	32.01	C
ATOM	818	SD	MET A	85	71.918	2.027	12.487	1.00	37.79	S
ATOM	819	CE	MET A	85	73.379	1.801	13.320	1.00	15.94	C

ATOM	820	N	ILE	A	86	70.471	3.892	17.481	1.00 13.92	N
ATOM	821	CA	ILE	A	86	70.760	3.893	18.912	1.00 12.58	C
ATOM	822	C	ILE	A	86	70.159	2.662	19.591	1.00 21.61	C
ATOM	823	O	ILE	A	86	70.813	1.981	20.362	1.00 18.68	O
ATOM	824	CB	ILE	A	86	70.225	5.189	19.606	1.00 11.84	C
ATOM	825	CG1	ILE	A	86	70.978	6.429	19.119	1.00 19.78	C
ATOM	826	CG2	ILE	A	86	70.435	5.132	21.112	1.00 6.59	C
ATOM	827	CD1	ILE	A	86	70.505	7.694	19.772	1.00 20.37	C
ATOM	828	N	GLU	A	87	68.893	2.383	19.316	1.00 18.78	N
ATOM	829	CA	GLU	A	87	68.263	1.237	19.930	1.00 14.00	C
ATOM	830	C	GLU	A	87	68.797	-0.116	19.454	1.00 15.93	C
ATOM	831	O	GLU	A	87	69.017	-0.991	20.268	1.00 11.04	O
ATOM	832	CB	GLU	A	87	66.734	1.324	19.900	1.00 14.89	C
ATOM	833	CG	GLU	A	87	66.085	1.327	18.538	1.00 28.96	C
ATOM	834	CD	GLU	A	87	64.635	1.922	18.544	1.00 11.12	C
ATOM	835	OE1	GLU	A	87	64.307	2.801	19.376	1.00 25.46	O
ATOM	836	OE2	GLU	A	87	63.845	1.547	17.663	1.00 29.87	O
ATOM	837	N	SER	A	88	69.054	-0.259	18.155	1.00 16.18	N
ATOM	838	CA	SER	A	88	69.650	-1.482	17.569	1.00 19.52	C
ATOM	839	C	SER	A	88	71.029	-1.792	18.160	1.00 22.54	C
ATOM	840	O	SER	A	88	71.313	-2.929	18.592	1.00 13.80	O
ATOM	841	CB	SER	A	88	69.815	-1.326	16.023	1.00 14.61	C
ATOM	842	OG	SER	A	88	68.551	-1.201	15.355	1.00 15.41	O
ATOM	843	N	ASN	A	89	71.884	-0.773	18.143	1.00 22.63	N
ATOM	844	CA	ASN	A	89	73.227	-0.869	18.693	1.00 27.23	C
ATOM	845	C	ASN	A	89	73.195	-1.363	20.134	1.00 21.34	C
ATOM	846	O	ASN	A	89	73.795	-2.384	20.476	1.00 23.68	O
ATOM	847	CB	ASN	A	89	73.980	0.487	18.597	1.00 13.71	C
ATOM	848	CG	ASN	A	89	74.440	0.825	17.168	1.00 20.40	C
ATOM	849	OD1	ASN	A	89	74.305	-0.006	16.255	1.00 14.93	O
ATOM	850	ND2	ASN	A	89	74.937	2.067	16.960	1.00 13.32	N
ATOM	851	N	ILE	A	90	72.488	-0.646	20.979	1.00 16.55	N
ATOM	852	CA	ILE	A	90	72.437	-1.014	22.398	1.00 21.51	C
ATOM	853	C	ILE	A	90	71.876	-2.421	22.729	1.00 26.50	C
ATOM	854	O	ILE	A	90	72.384	-3.159	23.590	1.00 19.71	O
ATOM	855	CB	ILE	A	90	71.670	0.070	23.233	1.00 13.32	C
ATOM	856	CG1	ILE	A	90	72.539	1.299	23.401	1.00 11.05	C
ATOM	857	CG2	ILE	A	90	71.371	-0.445	24.637	1.00 7.54	C
ATOM	858	CD1	ILE	A	90	71.749	2.597	23.668	1.00 20.71	C
ATOM	859	N	ILE	A	91	70.755	-2.733	22.114	1.00 14.98	N
ATOM	860	CA	ILE	A	91	70.047	-3.953	22.442	1.00 21.33	C
ATOM	861	C	ILE	A	91	70.927	-5.098	21.994	1.00 26.27	C
ATOM	862	O	ILE	A	91	71.211	-6.011	22.751	1.00 26.56	O
ATOM	863	CB	ILE	A	91	68.556	-3.930	21.814	1.00 20.39	C
ATOM	864	CG1	ILE	A	91	67.692	-2.886	22.552	1.00 13.51	C

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ATOM    865  CG2 ILE A  91      67.841  -5.316  21.845  1.00 11.31           C
ATOM    866  CD1 ILE A  91      66.320  -2.648  21.907  1.00 16.23           C
ATOM    867  N   HIS A  92      71.446  -4.983  20.785  1.00 24.12           N
ATOM    868  CA  HIS A  92      72.293  -6.015  20.243  1.00 26.71           C
ATOM    869  C   HIS A  92      73.609  -6.251  21.071  1.00 29.30           C
ATOM    870  O   HIS A  92      73.983  -7.366  21.443  1.00 18.58           O
ATOM    871  CB  HIS A  92      72.561  -5.682  18.775  1.00 22.23           C
ATOM    872  CG  HIS A  92      73.366  -6.720  18.077  1.00 26.32           C
ATOM    873  ND1 HIS A  92      72.798  -7.711  17.307  1.00 27.19           N
ATOM    874  CD2 HIS A  92      74.699  -6.978  18.106  1.00 21.95           C
ATOM    875  CE1 HIS A  92      73.755  -8.487  16.826  1.00 23.66           C
ATOM    876  NE2 HIS A  92      74.918  -8.062  17.296  1.00 17.36           N
ATOM    877  N   ALA A  93      74.328  -5.187  21.333  1.00 15.66           N
ATOM    878  CA  ALA A  93      75.530  -5.301  22.110  1.00 11.88           C
ATOM    879  C   ALA A  93      75.222  -5.900  23.512  1.00 28.78           C
ATOM    880  O   ALA A  93      75.912  -6.790  24.037  1.00 25.23           O
ATOM    881  CB  ALA A  93      76.139  -3.959  22.221  1.00  6.30           C
ATOM    882  N   ALA A  94      74.142  -5.442  24.113  1.00 18.82           N
ATOM    883  CA  ALA A  94      73.777  -5.971  25.399  1.00 15.61           C
ATOM    884  C   ALA A  94      73.593  -7.503  25.301  1.00 28.39           C
ATOM    885  O   ALA A  94      74.133  -8.263  26.099  1.00 21.67           O
ATOM    886  CB  ALA A  94      72.449  -5.279  25.911  1.00 18.46           C
ATOM    887  N   HIS A  95      72.814  -7.966  24.329  1.00 26.35           N
ATOM    888  CA  HIS A  95      72.551  -9.396  24.271  1.00 24.89           C
ATOM    889  C   HIS A  95      73.845 -10.176  24.140  1.00 22.81           C
ATOM    890  O   HIS A  95      74.077 -11.136  24.865  1.00 21.44           O
ATOM    891  CB  HIS A  95      71.571  -9.778  23.129  1.00 22.39           C
ATOM    892  CG  HIS A  95      71.554 -11.250  22.831  1.00 28.73           C
ATOM    893  ND1 HIS A  95      70.979 -12.182  23.682  1.00 22.83           N
ATOM    894  CD2 HIS A  95      72.159 -11.964  21.845  1.00 25.22           C
ATOM    895  CE1 HIS A  95      71.171 -13.397  23.196  1.00 22.72           C
ATOM    896  NE2 HIS A  95      71.911 -13.296  22.101  1.00 24.80           N
ATOM    897  N   GLN A  96      74.709  -9.658  23.281  1.00 19.97           N
ATOM    898  CA  GLN A  96      75.960 -10.299  22.917  1.00 22.27           C
ATOM    899  C   GLN A  96      76.877 -10.353  24.086  1.00 26.58           C
ATOM    900  O   GLN A  96      77.836 -11.093  24.088  1.00 24.17           O
ATOM    901  CB  GLN A  96      76.642  -9.492  21.818  1.00 23.38           C
ATOM    902  CG  GLN A  96      77.043 -10.299  20.596  1.00 61.06           C
ATOM    903  CD  GLN A  96      78.033  -9.557  19.675  1.00 75.83           C
ATOM    904  OE1 GLN A  96      78.999  -8.941  20.131  1.00 56.89           O
ATOM    905  NE2 GLN A  96      77.815  -9.668  18.366  1.00100.00           N
ATOM    906  N   ASN A  97      76.652  -9.500  25.060  1.00 22.15           N
ATOM    907  CA  ASN A  97      77.537  -9.536  26.208  1.00 14.74           C
ATOM    908  C   ASN A  97      76.732 -10.022  27.387  1.00 29.78           C
ATOM    909  O   ASN A  97      77.049  -9.762  28.564  1.00 27.09           O
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ATOM    910  CB  ASN A  97      78.241  -8.201  26.462  1.00 12.93           C
ATOM    911  CG  ASN A  97      79.260  -7.897  25.407  1.00 24.91           C
ATOM    912  OD1 ASN A  97      80.331  -8.518  25.375  1.00 57.17           O
ATOM    913  ND2 ASN A  97      78.839  -7.135  24.392  1.00 34.88           N
ATOM    914  N   ASP A  98      75.666 -10.732  27.055  1.00 27.98           N
ATOM    915  CA  ASP A  98      74.907 -11.361  28.089  1.00 29.25           C
ATOM    916  C   ASP A  98      74.400 -10.379  29.164  1.00 37.53           C
ATOM    917  O   ASP A  98      74.505 -10.634  30.367  1.00 36.42           O
ATOM    918  CB  ASP A  98      75.791 -12.450  28.700  1.00 36.37           C
ATOM    919  CG  ASP A  98      75.016 -13.712  29.053  1.00 88.62           C
ATOM    920  OD1 ASP A  98      73.775 -13.749  28.877  1.00 82.53           O
ATOM    921  OD2 ASP A  98      75.656 -14.670  29.542  1.00100.00           O
ATOM    922  N   VAL A  99      73.879  -9.235  28.730  1.00 27.13           N
ATOM    923  CA  VAL A  99      73.157  -8.351  29.635  1.00 21.57           C
ATOM    924  C   VAL A  99      71.706  -8.868  29.530  1.00 16.15           C
ATOM    925  O   VAL A  99      71.159  -9.088  28.422  1.00 19.47           O
ATOM    926  CB  VAL A  99      73.264  -6.900  29.206  1.00 24.18           C
ATOM    927  CG1 VAL A  99      72.517  -6.015  30.198  1.00 14.58           C
ATOM    928  CG2 VAL A  99      74.720  -6.515  29.225  1.00 30.10           C
ATOM    929  N   ASN A 100      71.149  -9.262  30.662  1.00 17.39           N
ATOM    930  CA  ASN A 100      69.852  -9.925  30.613  1.00 25.77           C
ATOM    931  C   ASN A 100      68.648  -9.034  30.910  1.00 24.95           C
ATOM    932  O   ASN A 100      67.498  -9.377  30.582  1.00 20.88           O
ATOM    933  CB  ASN A 100      69.846 -11.157  31.527  1.00 14.98           C
ATOM    934  CG  ASN A 100      68.724 -12.112  31.180  1.00 20.38           C
ATOM    935  OD1 ASN A 100      68.737 -12.709  30.100  1.00 29.59           O
ATOM    936  ND2 ASN A 100      67.716 -12.240  32.076  1.00 16.35           N
ATOM    937  N   LYS A 101      68.941  -7.923  31.584  1.00 17.91           N
ATOM    938  CA  LYS A 101      67.970  -6.916  31.994  1.00 25.43           C
ATOM    939  C   LYS A 101      68.107  -5.510  31.323  1.00 25.29           C
ATOM    940  O   LYS A 101      69.151  -4.850  31.377  1.00 19.88           O
ATOM    941  CB  LYS A 101      67.996  -6.807  33.521  1.00 29.28           C
ATOM    942  CG  LYS A 101      67.464  -8.054  34.205  1.00  9.31           C
ATOM    943  CD  LYS A 101      67.218  -7.719  35.668  1.00 38.93           C
ATOM    944  CE  LYS A 101      66.206  -6.569  35.885  1.00 13.38           C
ATOM    945  NZ  LYS A 101      64.750  -7.006  35.825  1.00 15.26           N
ATOM    946  N   LEU A 102      67.013  -5.043  30.732  1.00 22.22           N
ATOM    947  CA  LEU A 102      67.003  -3.744  30.092  1.00 15.40           C
ATOM    948  C   LEU A 102      65.612  -3.115  30.156  1.00 18.55           C
ATOM    949  O   LEU A 102      64.590  -3.811  30.102  1.00 18.92           O
ATOM    950  CB  LEU A 102      67.465  -3.898  28.636  1.00 11.23           C
ATOM    951  CG  LEU A 102      67.553  -2.711  27.651  1.00 15.51           C
ATOM    952  CD1 LEU A 102      68.628  -2.985  26.559  1.00  9.65           C
ATOM    953  CD2 LEU A 102      66.162  -2.407  26.995  1.00 13.10           C
ATOM    954  N   LEU A 103      65.595  -1.798  30.318  1.00 17.05           N
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ATOM	955	CA	LEU A 103	64.356	-1.036	30.265	1.00	16.23		C
ATOM	956	C	LEU A 103	64.346	-0.072	29.046	1.00	19.65		C
ATOM	957	O	LEU A 103	65.215	0.789	28.875	1.00	19.68		O
ATOM	958	CB	LEU A 103	64.099	-0.289	31.562	1.00	12.28		C
ATOM	959	CG	LEU A 103	62.686	0.259	31.594	1.00	14.13		C
ATOM	960	CD1	LEU A 103	61.645	-0.822	31.902	1.00	10.31		C
ATOM	961	CD2	LEU A 103	62.646	1.360	32.601	1.00	12.30		C
ATOM	962	N	PHE A 104	63.417	-0.333	28.140	1.00	16.41		N
ATOM	963	CA	PHE A 104	63.215	0.486	26.956	1.00	18.32		C
ATOM	964	C	PHE A 104	62.126	1.546	27.249	1.00	21.85		C
ATOM	965	O	PHE A 104	61.168	1.271	27.992	1.00	18.36		O
ATOM	966	CB	PHE A 104	62.796	-0.386	25.793	1.00	9.86		C
ATOM	967	CG	PHE A 104	62.732	0.348	24.508	1.00	16.81		C
ATOM	968	CD1	PHE A 104	63.894	0.714	23.840	1.00	25.04		C
ATOM	969	CD2	PHE A 104	61.511	0.795	24.005	1.00	22.59		C
ATOM	970	CE1	PHE A 104	63.836	1.448	22.619	1.00	31.26		C
ATOM	971	CE2	PHE A 104	61.449	1.535	22.814	1.00	15.59		C
ATOM	972	CZ	PHE A 104	62.625	1.895	22.139	1.00	11.67		C
ATOM	973	N	LEU A 105	62.341	2.762	26.734	1.00	20.33		N
ATOM	974	CA	LEU A 105	61.416	3.897	26.904	1.00	18.10		C
ATOM	975	C	LEU A 105	60.711	4.237	25.634	1.00	17.04		C
ATOM	976	O	LEU A 105	61.315	4.680	24.665	1.00	18.83		O
ATOM	977	CB	LEU A 105	62.178	5.146	27.214	1.00	17.49		C
ATOM	978	CG	LEU A 105	62.434	5.544	28.644	1.00	27.17		C
ATOM	979	CD1	LEU A 105	62.630	4.349	29.574	1.00	19.16		C
ATOM	980	CD2	LEU A 105	63.688	6.347	28.529	1.00	23.59		C
ATOM	981	N	GLY A 106	59.407	4.153	25.652	1.00	20.66		N
ATOM	982	CA	GLY A 106	58.679	4.536	24.455	1.00	21.03		C
ATOM	983	C	GLY A 106	58.080	5.935	24.597	1.00	17.32		C
ATOM	984	O	GLY A 106	58.690	6.858	25.113	1.00	26.89		O
ATOM	985	N	SER A 107	56.831	6.047	24.219	1.00	22.05		N
ATOM	986	CA	SER A 107	56.177	7.317	24.288	1.00	22.12		C
ATOM	987	C	SER A 107	54.686	7.212	23.923	1.00	19.06		C
ATOM	988	O	SER A 107	54.314	6.545	22.963	1.00	27.42		O
ATOM	989	CB	SER A 107	56.882	8.232	23.300	1.00	20.99		C
ATOM	990	OG	SER A 107	55.947	9.133	22.776	1.00	42.85		O
ATOM	991	N	SER A 108	53.826	7.890	24.671	1.00	27.42		N
ATOM	992	CA	SER A 108	52.382	7.947	24.339	1.00	26.43		C
ATOM	993	C	SER A 108	52.144	8.259	22.842	1.00	30.97		C
ATOM	994	O	SER A 108	51.242	7.709	22.217	1.00	33.46		O
ATOM	995	CB	SER A 108	51.710	9.072	25.144	1.00	19.87		C
ATOM	996	OG	SER A 108	52.495	10.266	25.071	1.00	70.88		O
ATOM	997	N	CYS A 109	52.927	9.180	22.278	1.00	24.73		N
ATOM	998	CA	CYS A 109	52.728	9.549	20.880	1.00	25.61		C
ATOM	999	C	CYS A 109	52.970	8.482	19.815	1.00	21.29		C

ATOM	1000	O	CYS A 109	52.967	8.737	18.623	1.00	31.31		O
ATOM	1001	CB	CYS A 109	53.369	10.899	20.544	1.00	39.55		C
ATOM	1002	SG	CYS A 109	55.153	11.077	20.847	1.00	49.24		S
ATOM	1003	N	ILE A 110	53.101	7.264	20.258	1.00	18.31		N
ATOM	1004	CA	ILE A 110	53.329	6.150	19.379	1.00	28.10		C
ATOM	1005	C	ILE A 110	51.977	5.489	19.082	1.00	15.38		C
ATOM	1006	O	ILE A 110	51.895	4.592	18.268	1.00	16.52		O
ATOM	1007	CB	ILE A 110	54.154	5.153	20.206	1.00	40.45		C
ATOM	1008	CG1	ILE A 110	55.604	5.510	20.136	1.00	39.02		C
ATOM	1009	CG2	ILE A 110	53.879	3.715	19.875	1.00	61.33		C
ATOM	1010	CD1	ILE A 110	56.429	4.338	20.549	1.00	82.74		C
ATOM	1011	N	TYR A 111	50.951	5.842	19.854	1.00	14.91		N
ATOM	1012	CA	TYR A 111	49.630	5.227	19.678	1.00	13.96		C
ATOM	1013	C	TYR A 111	48.956	5.831	18.459	1.00	20.40		C
ATOM	1014	O	TYR A 111	49.302	6.933	18.056	1.00	11.71		O
ATOM	1015	CB	TYR A 111	48.763	5.468	20.921	1.00	9.63		C
ATOM	1016	CG	TYR A 111	49.117	4.550	22.065	1.00	14.94		C
ATOM	1017	CD1	TYR A 111	48.985	3.159	21.938	1.00	9.73		C
ATOM	1018	CD2	TYR A 111	49.755	5.038	23.216	1.00	14.96		C
ATOM	1019	CE1	TYR A 111	49.344	2.273	23.014	1.00	6.53		C
ATOM	1020	CE2	TYR A 111	50.146	4.155	24.272	1.00	13.66		C
ATOM	1021	CZ	TYR A 111	49.873	2.787	24.171	1.00	17.86		C
ATOM	1022	OH	TYR A 111	50.266	1.927	25.157	1.00	11.37		O
ATOM	1023	N	PRO A 112	47.974	5.145	17.872	1.00	22.56		N
ATOM	1024	CA	PRO A 112	47.279	5.743	16.721	1.00	23.44		C
ATOM	1025	C	PRO A 112	46.589	7.111	16.988	1.00	17.82		C
ATOM	1026	O	PRO A 112	46.197	7.453	18.115	1.00	19.72		O
ATOM	1027	CB	PRO A 112	46.290	4.644	16.252	1.00	15.69		C
ATOM	1028	CG	PRO A 112	46.895	3.343	16.769	1.00	22.83		C
ATOM	1029	CD	PRO A 112	47.593	3.733	18.086	1.00	16.10		C
ATOM	1030	N	LYS A 113	46.418	7.866	15.915	1.00	19.48		N
ATOM	1031	CA	LYS A 113	45.793	9.167	15.994	1.00	23.50		C
ATOM	1032	C	LYS A 113	44.396	9.077	16.655	1.00	34.28		C
ATOM	1033	O	LYS A 113	44.046	9.887	17.524	1.00	46.14		O
ATOM	1034	CB	LYS A 113	45.675	9.735	14.593	1.00	30.04		C
ATOM	1035	CG	LYS A 113	46.219	11.124	14.477	1.00	43.78		C
ATOM	1036	CD	LYS A 113	45.381	11.941	13.515	1.00	100.00		C
ATOM	1037	CE	LYS A 113	44.361	12.836	14.250	1.00	100.00		C
ATOM	1038	NZ	LYS A 113	43.480	13.625	13.304	1.00	100.00		N
ATOM	1039	N	LEU A 114	43.591	8.103	16.250	1.00	26.33		N
ATOM	1040	CA	LEU A 114	42.267	7.957	16.833	1.00	20.65		C
ATOM	1041	C	LEU A 114	42.083	6.792	17.760	1.00	18.44		C
ATOM	1042	O	LEU A 114	41.002	6.278	17.918	1.00	34.04		O
ATOM	1043	CB	LEU A 114	41.194	8.002	15.780	1.00	24.37		C
ATOM	1044	CG	LEU A 114	41.587	9.122	14.830	1.00	40.86		C

ATOM 1045 CD1 LEU A 114 40.991 8.797 13.504 1.00 49.29 C
ATOM 1046 CD2 LEU A 114 41.139 10.512 15.300 1.00 26.85 C
ATOM 1047 N ALA A 115 43.103 6.473 18.527 1.00 29.00 N
ATOM 1048 CA ALA A 115 42.920 5.446 19.528 1.00 25.66 C
ATOM 1049 C ALA A 115 41.722 5.727 20.454 1.00 28.76 C
ATOM 1050 O ALA A 115 41.364 6.855 20.682 1.00 24.12 O
ATOM 1051 CB ALA A 115 44.177 5.272 20.326 1.00 16.86 C
ATOM 1052 N LYS A 116 41.137 4.675 20.998 1.00 30.21 N
ATOM 1053 CA LYS A 116 40.036 4.792 21.928 1.00 25.85 C
ATOM 1054 C LYS A 116 40.668 5.248 23.195 1.00 14.18 C
ATOM 1055 O LYS A 116 41.750 4.781 23.535 1.00 23.51 O
ATOM 1056 CB LYS A 116 39.369 3.415 22.116 1.00 22.05 C
ATOM 1057 CG LYS A 116 39.053 3.032 23.524 1.00 55.38 C
ATOM 1058 CD LYS A 116 37.963 1.955 23.549 1.00100.00 C
ATOM 1059 CE LYS A 116 37.120 1.953 24.835 1.00100.00 C
ATOM 1060 NZ LYS A 116 35.767 1.310 24.630 1.00100.00 N
ATOM 1061 N GLN A 117 40.021 6.208 23.856 1.00 18.23 N
ATOM 1062 CA GLN A 117 40.456 6.757 25.180 1.00 21.01 C
ATOM 1063 C GLN A 117 39.695 6.178 26.383 1.00 30.96 C
ATOM 1064 O GLN A 117 38.483 6.009 26.345 1.00 27.66 O
ATOM 1065 CB GLN A 117 40.215 8.263 25.179 1.00 11.32 C
ATOM 1066 CG GLN A 117 40.849 8.912 23.948 1.00 12.12 C
ATOM 1067 CD GLN A 117 42.404 8.823 23.954 1.00 24.10 C
ATOM 1068 OE1 GLN A 117 43.041 8.628 22.896 1.00 47.88 O
ATOM 1069 NE2 GLN A 117 43.001 8.953 25.131 1.00 14.24 N
ATOM 1070 N PRO A 118 40.374 5.992 27.499 1.00 30.02 N
ATOM 1071 CA PRO A 118 41.826 6.194 27.655 1.00 26.44 C
ATOM 1072 C PRO A 118 42.450 5.050 26.899 1.00 24.37 C
ATOM 1073 O PRO A 118 41.792 4.027 26.726 1.00 25.34 O
ATOM 1074 CB PRO A 118 42.055 5.994 29.167 1.00 23.89 C
ATOM 1075 CG PRO A 118 40.847 5.240 29.654 1.00 23.20 C
ATOM 1076 CD PRO A 118 39.695 5.519 28.709 1.00 15.79 C
ATOM 1077 N MET A 119 43.684 5.228 26.432 1.00 16.00 N
ATOM 1078 CA MET A 119 44.372 4.215 25.644 1.00 10.80 C
ATOM 1079 C MET A 119 45.062 3.083 26.444 1.00 23.61 C
ATOM 1080 O MET A 119 46.013 3.281 27.209 1.00 18.02 O
ATOM 1081 CB MET A 119 45.384 4.894 24.791 1.00 13.52 C
ATOM 1082 CG MET A 119 44.801 6.014 23.989 1.00 18.52 C
ATOM 1083 SD MET A 119 46.157 7.054 23.271 1.00 26.27 S
ATOM 1084 CE MET A 119 46.264 6.524 21.845 1.00 33.79 C
ATOM 1085 N ALA A 120 44.559 1.875 26.271 1.00 26.64 N
ATOM 1086 CA ALA A 120 45.177 0.712 26.884 1.00 29.17 C
ATOM 1087 C ALA A 120 46.356 0.308 25.984 1.00 23.21 C
ATOM 1088 O ALA A 120 46.439 0.759 24.833 1.00 20.19 O
ATOM 1089 CB ALA A 120 44.169 -0.419 26.944 1.00 26.02 C

ATOM 1090 N GLU A 121 47.238 -0.553 26.507 1.00 12.30 N
ATOM 1091 CA GLU A 121 48.427 -1.009 25.788 1.00 9.45 C
ATOM 1092 C GLU A 121 48.070 -1.697 24.450 1.00 11.68 C
ATOM 1093 O GLU A 121 48.828 -1.670 23.450 1.00 14.84 O
ATOM 1094 CB GLU A 121 49.321 -1.883 26.715 1.00 16.74 C
ATOM 1095 CG GLU A 121 50.132 -1.122 27.763 1.00 18.14 C
ATOM 1096 CD GLU A 121 49.458 -1.000 29.137 1.00 13.00 C
ATOM 1097 OE1 GLU A 121 48.252 -1.294 29.276 1.00 20.79 O
ATOM 1098 OE2 GLU A 121 50.123 -0.521 30.080 1.00 17.86 O
ATOM 1099 N SER A 122 46.887 -2.273 24.409 1.00 11.79 N
ATOM 1100 CA SER A 122 46.427 -2.977 23.218 1.00 12.16 C
ATOM 1101 C SER A 122 46.030 -2.058 22.100 1.00 11.70 C
ATOM 1102 O SER A 122 45.717 -2.529 21.010 1.00 13.91 O
ATOM 1103 CB SER A 122 45.186 -3.781 23.568 1.00 21.50 C
ATOM 1104 OG SER A 122 44.143 -2.908 23.976 1.00 28.52 O
ATOM 1105 N GLU A 123 46.041 -0.754 22.341 1.00 14.65 N
ATOM 1106 CA GLU A 123 45.783 0.202 21.243 1.00 17.15 C
ATOM 1107 C GLU A 123 46.959 0.313 20.240 1.00 11.48 C
ATOM 1108 O GLU A 123 46.821 0.844 19.141 1.00 11.19 O
ATOM 1109 CB GLU A 123 45.481 1.600 21.805 1.00 21.66 C
ATOM 1110 CG GLU A 123 44.127 1.694 22.523 1.00 24.68 C
ATOM 1111 CD GLU A 123 42.984 1.374 21.585 1.00 35.56 C
ATOM 1112 OE1 GLU A 123 43.019 1.865 20.426 1.00 41.73 O
ATOM 1113 OE2 GLU A 123 42.158 0.497 21.940 1.00100.00 O
ATOM 1114 N LEU A 124 48.134 -0.185 20.618 1.00 14.02 N
ATOM 1115 CA LEU A 124 49.296 -0.082 19.740 1.00 15.32 C
ATOM 1116 C LEU A 124 49.082 -0.754 18.458 1.00 17.76 C
ATOM 1117 O LEU A 124 48.752 -1.917 18.445 1.00 18.91 O
ATOM 1118 CB LEU A 124 50.564 -0.680 20.362 1.00 18.07 C
ATOM 1119 CG LEU A 124 51.922 -0.222 19.803 1.00 21.52 C
ATOM 1120 CD1 LEU A 124 52.080 1.258 20.117 1.00 20.35 C
ATOM 1121 CD2 LEU A 124 53.042 -0.919 20.550 1.00 14.07 C
ATOM 1122 N LEU A 125 49.514 -0.071 17.409 1.00 18.44 N
ATOM 1123 CA LEU A 125 49.445 -0.564 16.052 1.00 19.92 C
ATOM 1124 C LEU A 125 48.034 -0.754 15.509 1.00 25.56 C
ATOM 1125 O LEU A 125 47.854 -1.188 14.364 1.00 18.26 O
ATOM 1126 CB LEU A 125 50.355 -1.800 15.840 1.00 20.79 C
ATOM 1127 CG LEU A 125 51.890 -1.511 15.778 1.00 17.21 C
ATOM 1128 CD1 LEU A 125 52.744 -2.649 16.316 1.00 19.95 C
ATOM 1129 CD2 LEU A 125 52.334 -1.219 14.338 1.00 5.81 C
ATOM 1130 N GLN A 126 47.027 -0.327 16.276 1.00 21.97 N
ATOM 1131 CA GLN A 126 45.652 -0.504 15.790 1.00 19.97 C
ATOM 1132 C GLN A 126 45.213 0.447 14.724 1.00 28.31 C
ATOM 1133 O GLN A 126 44.076 0.391 14.293 1.00 47.49 O
ATOM 1134 CB GLN A 126 44.652 -0.404 16.911 1.00 19.87 C

ATOM 1135 CG GLN A 126 44.949 -1.312 18.048 1.00 18.39 C
ATOM 1136 CD GLN A 126 44.319 -2.626 17.835 1.00 66.80 C
ATOM 1137 OE1 GLN A 126 44.064 -3.376 18.792 1.00 40.75 O
ATOM 1138 NE2 GLN A 126 44.015 -2.952 16.565 1.00 71.74 N
ATOM 1139 N GLY A 127 46.080 1.330 14.270 1.00 28.29 N
ATOM 1140 CA GLY A 127 45.627 2.260 13.252 1.00 23.31 C
ATOM 1141 C GLY A 127 46.662 3.315 12.953 1.00 22.90 C
ATOM 1142 O GLY A 127 47.755 3.254 13.474 1.00 25.30 O
ATOM 1143 N THR A 128 46.311 4.219 12.046 1.00 19.51 N
ATOM 1144 CA THR A 128 47.149 5.314 11.588 1.00 22.12 C
ATOM 1145 C THR A 128 47.705 6.219 12.695 1.00 22.60 C
ATOM 1146 O THR A 128 47.061 6.461 13.731 1.00 18.58 O
ATOM 1147 CB THR A 128 46.392 6.182 10.544 1.00 35.98 C
ATOM 1148 OG1 THR A 128 46.533 5.594 9.239 1.00 58.05 O
ATOM 1149 CG2 THR A 128 46.942 7.639 10.542 1.00 43.41 C
ATOM 1150 N LEU A 129 48.907 6.715 12.425 1.00 18.32 N
ATOM 1151 CA LEU A 129 49.674 7.534 13.356 1.00 16.76 C
ATOM 1152 C LEU A 129 49.504 8.959 12.967 1.00 4.89 C
ATOM 1153 O LEU A 129 49.232 9.260 11.814 1.00 16.14 O
ATOM 1154 CB LEU A 129 51.205 7.191 13.261 1.00 17.91 C
ATOM 1155 CG LEU A 129 51.769 5.804 13.752 1.00 18.21 C
ATOM 1156 CD1 LEU A 129 53.132 5.379 13.193 1.00 12.12 C
ATOM 1157 CD2 LEU A 129 51.683 5.532 15.251 1.00 3.89 C
ATOM 1158 N GLU A 130 49.816 9.827 13.917 1.00 10.23 N
ATOM 1159 CA GLU A 130 49.912 11.268 13.691 1.00 13.22 C
ATOM 1160 C GLU A 130 51.128 11.544 12.775 1.00 23.44 C
ATOM 1161 O GLU A 130 52.249 11.162 13.090 1.00 21.23 O
ATOM 1162 CB GLU A 130 50.150 11.979 15.035 1.00 18.48 C
ATOM 1163 CG GLU A 130 50.754 13.376 14.886 1.00 77.44 C
ATOM 1164 CD GLU A 130 49.833 14.328 14.121 1.00100.00 C
ATOM 1165 OE1 GLU A 130 48.588 14.205 14.340 1.00 36.19 O
ATOM 1166 OE2 GLU A 130 50.347 15.161 13.295 1.00 21.03 O
ATOM 1167 N PRO A 131 50.920 12.219 11.648 1.00 21.35 N
ATOM 1168 CA PRO A 131 52.023 12.409 10.731 1.00 14.78 C
ATOM 1169 C PRO A 131 53.201 13.132 11.265 1.00 14.98 C
ATOM 1170 O PRO A 131 54.325 12.847 10.853 1.00 20.99 O
ATOM 1171 CB PRO A 131 51.413 13.154 9.552 1.00 14.76 C
ATOM 1172 CG PRO A 131 50.071 13.485 9.949 1.00 20.99 C
ATOM 1173 CD PRO A 131 49.641 12.626 11.047 1.00 17.25 C
ATOM 1174 N THR A 132 52.986 14.095 12.159 1.00 18.77 N
ATOM 1175 CA THR A 132 54.131 14.838 12.689 1.00 16.44 C
ATOM 1176 C THR A 132 55.102 13.951 13.408 1.00 21.91 C
ATOM 1177 O THR A 132 56.317 14.088 13.234 1.00 24.17 O
ATOM 1178 CB THR A 132 53.716 15.907 13.606 1.00 23.45 C
ATOM 1179 OG1 THR A 132 52.976 16.883 12.850 1.00 31.15 O

ATOM	1180	CG2	THR	A	132	54.969	16.519	14.341	1.00	9.28	C
ATOM	1181	N	ASN	A	133	54.551	12.970	14.122	1.00	28.59	N
ATOM	1182	CA	ASN	A	133	55.359	12.007	14.875	1.00	26.38	C
ATOM	1183	C	ASN	A	133	55.666	10.682	14.207	1.00	14.85	C
ATOM	1184	O	ASN	A	133	56.446	9.884	14.755	1.00	18.67	O
ATOM	1185	CB	ASN	A	133	54.661	11.699	16.168	1.00	23.70	C
ATOM	1186	CG	ASN	A	133	54.480	12.894	16.968	1.00	50.55	C
ATOM	1187	OD1	ASN	A	133	53.354	13.272	17.252	1.00	40.07	O
ATOM	1188	ND2	ASN	A	133	55.568	13.638	17.163	1.00	40.36	N
ATOM	1189	N	GLU	A	134	55.100	10.469	13.022	1.00	9.98	N
ATOM	1190	CA	GLU	A	134	55.237	9.210	12.365	1.00	9.66	C
ATOM	1191	C	GLU	A	134	56.648	8.530	12.274	1.00	13.86	C
ATOM	1192	O	GLU	A	134	56.814	7.388	12.706	1.00	22.89	O
ATOM	1193	CB	GLU	A	134	54.448	9.200	11.070	1.00	17.55	C
ATOM	1194	CG	GLU	A	134	54.750	7.930	10.227	1.00	20.89	C
ATOM	1195	CD	GLU	A	134	53.926	7.868	8.970	1.00	13.59	C
ATOM	1196	OE1	GLU	A	134	52.678	7.738	9.085	1.00	35.28	O
ATOM	1197	OE2	GLU	A	134	54.497	8.048	7.869	1.00	13.44	O
ATOM	1198	N	PRO	A	135	57.680	9.222	11.789	1.00	15.72	N
ATOM	1199	CA	PRO	A	135	59.014	8.600	11.699	1.00	18.91	C
ATOM	1200	C	PRO	A	135	59.544	8.174	13.073	1.00	18.68	C
ATOM	1201	O	PRO	A	135	60.072	7.069	13.271	1.00	15.69	O
ATOM	1202	CB	PRO	A	135	59.896	9.755	11.169	1.00	13.84	C
ATOM	1203	CG	PRO	A	135	59.036	10.514	10.350	1.00	9.78	C
ATOM	1204	CD	PRO	A	135	57.594	10.395	10.908	1.00	14.43	C
ATOM	1205	N	TYR	A	136	59.449	9.117	13.994	1.00	8.64	N
ATOM	1206	CA	TYR	A	136	59.873	8.915	15.324	1.00	13.27	C
ATOM	1207	C	TYR	A	136	59.056	7.728	15.907	1.00	16.84	C
ATOM	1208	O	TYR	A	136	59.578	6.903	16.658	1.00	12.90	O
ATOM	1209	CB	TYR	A	136	59.604	10.234	16.100	1.00	15.51	C
ATOM	1210	CG	TYR	A	136	59.912	10.168	17.614	1.00	18.26	C
ATOM	1211	CD1	TYR	A	136	61.200	10.062	18.072	1.00	20.53	C
ATOM	1212	CD2	TYR	A	136	58.904	10.150	18.568	1.00	17.38	C
ATOM	1213	CE1	TYR	A	136	61.484	9.959	19.440	1.00	30.44	C
ATOM	1214	CE2	TYR	A	136	59.184	10.084	19.953	1.00	9.85	C
ATOM	1215	CZ	TYR	A	136	60.476	9.949	20.377	1.00	20.65	C
ATOM	1216	OH	TYR	A	136	60.792	9.873	21.734	1.00	24.41	O
ATOM	1217	N	ALA	A	137	57.760	7.687	15.638	1.00	7.19	N
ATOM	1218	CA	ALA	A	137	56.923	6.633	16.227	1.00	12.68	C
ATOM	1219	C	ALA	A	137	57.345	5.265	15.737	1.00	15.21	C
ATOM	1220	O	ALA	A	137	57.425	4.272	16.488	1.00	14.58	O
ATOM	1221	CB	ALA	A	137	55.517	6.849	15.871	1.00	11.40	C
ATOM	1222	N	ILE	A	138	57.567	5.213	14.447	1.00	8.93	N
ATOM	1223	CA	ILE	A	138	57.954	3.971	13.831	1.00	11.77	C
ATOM	1224	C	ILE	A	138	59.246	3.494	14.492	1.00	16.20	C

ATOM 1225 O ILE A 138 59.307 2.377 14.970 1.00 13.79 O
ATOM 1226 CB ILE A 138 58.064 4.172 12.316 1.00 17.85 C
ATOM 1227 CG1 ILE A 138 56.680 4.473 11.757 1.00 28.21 C
ATOM 1228 CG2 ILE A 138 58.674 2.986 11.602 1.00 9.81 C
ATOM 1229 CD1 ILE A 138 55.695 3.376 11.970 1.00 18.17 C
ATOM 1230 N ALA A 139 60.243 4.361 14.625 1.00 11.54 N
ATOM 1231 CA ALA A 139 61.494 3.937 15.288 1.00 13.22 C
ATOM 1232 C ALA A 139 61.256 3.364 16.675 1.00 18.73 C
ATOM 1233 O ALA A 139 61.791 2.318 17.031 1.00 20.44 O
ATOM 1234 CB ALA A 139 62.434 5.073 15.390 1.00 13.62 C
ATOM 1235 N LYS A 140 60.397 4.033 17.448 1.00 16.36 N
ATOM 1236 CA LYS A 140 60.083 3.600 18.815 1.00 15.14 C
ATOM 1237 C LYS A 140 59.392 2.262 18.824 1.00 15.18 C
ATOM 1238 O LYS A 140 59.824 1.346 19.475 1.00 21.42 O
ATOM 1239 CB LYS A 140 59.193 4.606 19.525 1.00 17.86 C
ATOM 1240 CG LYS A 140 59.925 5.806 20.152 1.00 21.11 C
ATOM 1241 CD LYS A 140 61.208 5.478 20.958 1.00 16.75 C
ATOM 1242 CE LYS A 140 61.664 6.735 21.835 1.00 10.06 C
ATOM 1243 NZ LYS A 140 62.688 6.496 22.921 1.00 14.40 N
ATOM 1244 N ILE A 141 58.356 2.116 18.027 1.00 11.49 N
ATOM 1245 CA ILE A 141 57.703 0.828 17.977 1.00 17.92 C
ATOM 1246 C ILE A 141 58.729 -0.282 17.577 1.00 13.46 C
ATOM 1247 O ILE A 141 58.730 -1.374 18.148 1.00 13.92 O
ATOM 1248 CB ILE A 141 56.497 0.925 17.019 1.00 22.59 C
ATOM 1249 CG1 ILE A 141 55.466 1.906 17.557 1.00 17.61 C
ATOM 1250 CG2 ILE A 141 55.863 -0.411 16.700 1.00 10.49 C
ATOM 1251 CD1 ILE A 141 54.530 2.327 16.449 1.00 13.43 C
ATOM 1252 N ALA A 142 59.637 0.028 16.650 1.00 10.29 N
ATOM 1253 CA ALA A 142 60.657 -0.931 16.228 1.00 7.15 C
ATOM 1254 C ALA A 142 61.456 -1.301 17.456 1.00 16.58 C
ATOM 1255 O ALA A 142 61.839 -2.454 17.621 1.00 13.04 O
ATOM 1256 CB ALA A 142 61.604 -0.288 15.130 1.00 4.44 C
ATOM 1257 N GLY A 143 61.703 -0.307 18.316 1.00 9.56 N
ATOM 1258 CA GLY A 143 62.448 -0.525 19.527 1.00 5.15 C
ATOM 1259 C GLY A 143 61.770 -1.555 20.430 1.00 16.36 C
ATOM 1260 O GLY A 143 62.392 -2.482 20.967 1.00 14.11 O
ATOM 1261 N ILE A 144 60.476 -1.418 20.564 1.00 20.33 N
ATOM 1262 CA ILE A 144 59.725 -2.314 21.407 1.00 15.35 C
ATOM 1263 C ILE A 144 59.706 -3.732 20.859 1.00 19.84 C
ATOM 1264 O ILE A 144 59.836 -4.700 21.608 1.00 17.93 O
ATOM 1265 CB ILE A 144 58.317 -1.819 21.559 1.00 10.60 C
ATOM 1266 CG1 ILE A 144 58.311 -0.610 22.516 1.00 9.80 C
ATOM 1267 CG2 ILE A 144 57.410 -2.928 22.122 1.00 9.60 C
ATOM 1268 CD1 ILE A 144 57.022 0.076 22.517 1.00 18.32 C
ATOM 1269 N LYS A 145 59.520 -3.841 19.556 1.00 7.20 N

ATOM	1270	CA	LYS A 145	59.459	-5.139	18.926	1.00	7.64	C
ATOM	1271	C	LYS A 145	60.840	-5.788	18.931	1.00	15.32	C
ATOM	1272	O	LYS A 145	60.923	-6.989	18.981	1.00	14.76	O
ATOM	1273	CB	LYS A 145	58.891	-5.001	17.516	1.00	11.25	C
ATOM	1274	CG	LYS A 145	57.414	-4.581	17.489	1.00	12.13	C
ATOM	1275	CD	LYS A 145	56.642	-5.434	18.495	1.00	25.23	C
ATOM	1276	CE	LYS A 145	55.189	-4.995	18.692	1.00	13.64	C
ATOM	1277	NZ	LYS A 145	54.441	-6.111	19.392	1.00	11.94	N
ATOM	1278	N	LEU A 146	61.934	-5.011	18.986	1.00	26.98	N
ATOM	1279	CA	LEU A 146	63.261	-5.642	19.167	1.00	19.72	C
ATOM	1280	C	LEU A 146	63.262	-6.316	20.542	1.00	18.20	C
ATOM	1281	O	LEU A 146	63.590	-7.511	20.703	1.00	19.86	O
ATOM	1282	CB	LEU A 146	64.398	-4.618	19.150	1.00	13.56	C
ATOM	1283	CG	LEU A 146	64.895	-4.258	17.759	1.00	21.84	C
ATOM	1284	CD1	LEU A 146	65.672	-2.945	17.817	1.00	17.94	C
ATOM	1285	CD2	LEU A 146	65.745	-5.397	17.102	1.00	16.10	C
ATOM	1286	N	CYS A 147	62.931	-5.523	21.548	1.00	7.91	N
ATOM	1287	CA	CYS A 147	62.875	-6.064	22.893	1.00	9.14	C
ATOM	1288	C	CYS A 147	62.072	-7.378	22.945	1.00	22.72	C
ATOM	1289	O	CYS A 147	62.568	-8.401	23.383	1.00	16.90	O
ATOM	1290	CB	CYS A 147	62.232	-5.058	23.809	1.00	12.63	C
ATOM	1291	SG	CYS A 147	63.411	-3.823	24.316	1.00	15.02	S
ATOM	1292	N	GLU A 148	60.823	-7.352	22.508	1.00	20.03	N
ATOM	1293	CA	GLU A 148	60.016	-8.555	22.567	1.00	16.09	C
ATOM	1294	C	GLU A 148	60.685	-9.715	21.802	1.00	22.61	C
ATOM	1295	O	GLU A 148	60.651	-10.888	22.226	1.00	12.05	O
ATOM	1296	CB	GLU A 148	58.597	-8.268	22.046	1.00	14.66	C
ATOM	1297	CG	GLU A 148	57.864	-7.189	22.840	1.00	11.45	C
ATOM	1298	CD	GLU A 148	56.471	-6.821	22.277	1.00	11.75	C
ATOM	1299	OE1	GLU A 148	56.117	-7.055	21.080	1.00	11.65	O
ATOM	1300	OE2	GLU A 148	55.728	-6.231	23.081	1.00	22.56	O
ATOM	1301	N	SER A 149	61.368	-9.377	20.715	1.00	15.57	N
ATOM	1302	CA	SER A 149	61.938	-10.428	19.887	1.00	10.21	C
ATOM	1303	C	SER A 149	63.040	-11.245	20.502	1.00	15.83	C
ATOM	1304	O	SER A 149	63.102	-12.458	20.291	1.00	12.72	O
ATOM	1305	CB	SER A 149	62.270	-9.936	18.488	1.00	9.44	C
ATOM	1306	OG	SER A 149	61.053	-9.650	17.782	1.00	15.91	O
ATOM	1307	N	TYR A 150	63.910	-10.546	21.224	1.00	18.44	N
ATOM	1308	CA	TYR A 150	65.065	-11.100	21.948	1.00	20.50	C
ATOM	1309	C	TYR A 150	64.514	-11.848	23.158	1.00	21.87	C
ATOM	1310	O	TYR A 150	64.939	-12.949	23.486	1.00	31.39	O
ATOM	1311	CB	TYR A 150	66.005	-9.950	22.425	1.00	13.71	C
ATOM	1312	CG	TYR A 150	66.994	-9.509	21.365	1.00	14.13	C
ATOM	1313	CD1	TYR A 150	66.611	-8.673	20.317	1.00	14.64	C
ATOM	1314	CD2	TYR A 150	68.288	-10.000	21.360	1.00	18.32	C

ATOM 1315 CE1 TYR A 150 67.487 -8.390 19.278 1.00 11.91 C
ATOM 1316 CE2 TYR A 150 69.198 -9.682 20.345 1.00 11.10 C
ATOM 1317 CZ TYR A 150 68.804 -8.900 19.326 1.00 20.95 C
ATOM 1318 OH TYR A 150 69.739 -8.685 18.333 1.00 27.73 O
ATOM 1319 N ASN A 151 63.536 -11.249 23.801 1.00 14.83 N
ATOM 1320 CA ASN A 151 62.903 -11.889 24.937 1.00 23.62 C
ATOM 1321 C ASN A 151 62.417 -13.244 24.410 1.00 28.53 C
ATOM 1322 O ASN A 151 62.630 -14.248 25.072 1.00 25.89 O
ATOM 1323 CB ASN A 151 61.655 -11.113 25.439 1.00 20.95 C
ATOM 1324 CG ASN A 151 61.988 -9.867 26.284 1.00 15.07 C
ATOM 1325 OD1 ASN A 151 61.126 -9.020 26.466 1.00 26.72 O
ATOM 1326 ND2 ASN A 151 63.231 -9.709 26.700 1.00 6.31 N
ATOM 1327 N ARG A 152 61.731 -13.249 23.259 1.00 19.91 N
ATOM 1328 CA ARG A 152 61.129 -14.465 22.687 1.00 17.62 C
ATOM 1329 C ARG A 152 62.090 -15.523 22.188 1.00 21.34 C
ATOM 1330 O ARG A 152 61.959 -16.687 22.542 1.00 15.44 O
ATOM 1331 CB ARG A 152 60.086 -14.148 21.610 1.00 15.30 C
ATOM 1332 CG ARG A 152 58.672 -13.754 22.157 1.00 17.22 C
ATOM 1333 CD ARG A 152 57.652 -13.297 21.049 1.00 9.11 C
ATOM 1334 NE ARG A 152 57.161 -14.419 20.241 1.00 21.05 N
ATOM 1335 CZ ARG A 152 57.159 -14.447 18.912 1.00 28.61 C
ATOM 1336 NH1 ARG A 152 57.590 -13.387 18.221 1.00 21.98 N
ATOM 1337 NH2 ARG A 152 56.717 -15.528 18.262 1.00 26.11 N
ATOM 1338 N GLN A 153 63.098 -15.104 21.434 1.00 16.54 N
ATOM 1339 CA GLN A 153 64.044 -16.036 20.842 1.00 9.74 C
ATOM 1340 C GLN A 153 65.082 -16.443 21.807 1.00 16.70 C
ATOM 1341 O GLN A 153 65.529 -17.545 21.763 1.00 24.35 O
ATOM 1342 CB GLN A 153 64.789 -15.372 19.714 1.00 8.99 C
ATOM 1343 CG GLN A 153 65.935 -16.225 19.116 1.00 4.63 C
ATOM 1344 CD GLN A 153 66.315 -15.637 17.762 1.00 14.17 C
ATOM 1345 OE1 GLN A 153 65.611 -14.763 17.254 1.00 12.53 O
ATOM 1346 NE2 GLN A 153 67.466 -16.024 17.228 1.00 13.38 N
ATOM 1347 N TYR A 154 65.566 -15.518 22.608 1.00 14.35 N
ATOM 1348 CA TYR A 154 66.677 -15.839 23.483 1.00 12.16 C
ATOM 1349 C TYR A 154 66.323 -15.930 24.954 1.00 19.06 C
ATOM 1350 O TYR A 154 67.185 -16.207 25.777 1.00 25.59 O
ATOM 1351 CB TYR A 154 67.829 -14.816 23.326 1.00 16.89 C
ATOM 1352 CG TYR A 154 68.418 -14.733 21.943 1.00 17.53 C
ATOM 1353 CD1 TYR A 154 69.259 -15.726 21.467 1.00 18.91 C
ATOM 1354 CD2 TYR A 154 68.080 -13.712 21.091 1.00 13.97 C
ATOM 1355 CE1 TYR A 154 69.782 -15.686 20.190 1.00 10.98 C
ATOM 1356 CE2 TYR A 154 68.621 -13.639 19.806 1.00 23.81 C
ATOM 1357 CZ TYR A 154 69.488 -14.634 19.380 1.00 23.08 C
ATOM 1358 OH TYR A 154 70.002 -14.619 18.118 1.00 23.87 O
ATOM 1359 N GLY A 155 65.080 -15.686 25.313 1.00 12.08 N

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ATOM   1360  CA  GLY A 155      64.747 -15.702  26.731  1.00 15.80           C
ATOM   1361  C   GLY A 155      65.323 -14.498  27.580  1.00 33.97           C
ATOM   1362  O   GLY A 155      65.491 -14.640  28.789  1.00 25.76           O
ATOM   1363  N   ARG A 156      65.564 -13.318  26.981  1.00 25.91           N
ATOM   1364  CA  ARG A 156      66.066 -12.146  27.734  1.00 14.13           C
ATOM   1365  C   ARG A 156      64.971 -11.486  28.581  1.00 16.23           C
ATOM   1366  O   ARG A 156      63.802 -11.919  28.583  1.00 22.61           O
ATOM   1367  CB  ARG A 156      66.601 -11.124  26.750  1.00 13.16           C
ATOM   1368  CG  ARG A 156      67.875 -11.570  26.099  1.00 15.18           C
ATOM   1369  CD  ARG A 156      68.930 -11.418  27.121  1.00 26.42           C
ATOM   1370  NE  ARG A 156      70.200 -11.912  26.633  1.00 21.25           N
ATOM   1371  CZ  ARG A 156      71.092 -12.555  27.386  1.00 42.25           C
ATOM   1372  NH1 ARG A 156      70.870 -12.795  28.679  1.00 20.02           N
ATOM   1373  NH2 ARG A 156      72.221 -12.966  26.843  1.00 20.88           N
ATOM   1374  N   ASP A 157      65.343 -10.446  29.321  1.00 16.00           N
ATOM   1375  CA  ASP A 157      64.370  -9.749  30.166  1.00 16.20           C
ATOM   1376  C   ASP A 157      64.444  -8.245  29.841  1.00 19.20           C
ATOM   1377  O   ASP A 157      64.865  -7.429  30.650  1.00 10.71           O
ATOM   1378  CB  ASP A 157      64.609 -10.061  31.652  1.00 16.50           C
ATOM   1379  CG  ASP A 157      63.489  -9.560  32.566  1.00 26.45           C
ATOM   1380  OD1 ASP A 157      62.433  -9.060  32.108  1.00 26.82           O
ATOM   1381  OD2 ASP A 157      63.673  -9.653  33.784  1.00 21.88           O
ATOM   1382  N   TYR A 158      64.038  -7.921  28.620  1.00 19.41           N
ATOM   1383  CA  TYR A 158      64.099  -6.564  28.083  1.00 18.96           C
ATOM   1384  C   TYR A 158      62.688  -5.977  28.127  1.00 22.62           C
ATOM   1385  O   TYR A 158      61.854  -6.296  27.282  1.00 10.12           O
ATOM   1386  CB  TYR A 158      64.562  -6.661  26.631  1.00 16.34           C
ATOM   1387  CG  TYR A 158      65.982  -7.166  26.484  1.00 12.04           C
ATOM   1388  CD1 TYR A 158      66.789  -7.415  27.621  1.00 13.76           C
ATOM   1389  CD2 TYR A 158      66.544  -7.349  25.218  1.00 16.35           C
ATOM   1390  CE1 TYR A 158      68.135  -7.786  27.482  1.00  8.18           C
ATOM   1391  CE2 TYR A 158      67.886  -7.732  25.060  1.00 13.73           C
ATOM   1392  CZ  TYR A 158      68.676  -7.942  26.186  1.00 24.45           C
ATOM   1393  OH  TYR A 158      69.993  -8.338  25.997  1.00 14.36           O
ATOM   1394  N   ARG A 159      62.423  -5.200  29.175  1.00 23.53           N
ATOM   1395  CA  ARG A 159      61.105  -4.603  29.483  1.00 21.15           C
ATOM   1396  C   ARG A 159      60.930  -3.172  28.878  1.00 23.55           C
ATOM   1397  O   ARG A 159      61.911  -2.566  28.424  1.00 18.12           O
ATOM   1398  CB  ARG A 159      60.891  -4.608  31.034  1.00 21.68           C
ATOM   1399  CG  ARG A 159      60.986  -6.029  31.722  1.00 16.41           C
ATOM   1400  CD  ARG A 159      61.135  -6.052  33.233  1.00 18.10           C
ATOM   1401  NE  ARG A 159      61.305  -7.402  33.772  1.00 19.25           N
ATOM   1402  CZ  ARG A 159      61.164  -7.720  35.058  1.00 36.67           C
ATOM   1403  NH1 ARG A 159      60.886  -6.776  35.962  1.00 15.32           N
ATOM   1404  NH2 ARG A 159      61.309  -8.986  35.448  1.00 11.79           N
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ATOM	1405	N	SER A 160	59.689	-2.661	28.859	1.00	24.44		N
ATOM	1406	CA	SER A 160	59.312	-1.393	28.200	1.00	21.59		C
ATOM	1407	C	SER A 160	58.242	-0.577	28.950	1.00	25.07		C
ATOM	1408	O	SER A 160	57.257	-1.127	29.454	1.00	17.02		O
ATOM	1409	CB	SER A 160	58.719	-1.747	26.797	1.00	13.05		C
ATOM	1410	OG	SER A 160	59.782	-1.897	25.885	1.00	37.57		O
ATOM	1411	N	VAL A 161	58.378	0.742	28.927	1.00	21.01		N
ATOM	1412	CA	VAL A 161	57.369	1.644	29.509	1.00	9.70		C
ATOM	1413	C	VAL A 161	57.068	2.747	28.504	1.00	16.77		C
ATOM	1414	O	VAL A 161	57.955	3.149	27.729	1.00	16.33		O
ATOM	1415	CB	VAL A 161	57.806	2.248	30.862	1.00	17.94		C
ATOM	1416	CG1	VAL A 161	57.873	1.185	31.984	1.00	16.16		C
ATOM	1417	CG2	VAL A 161	59.137	2.992	30.750	1.00	21.10		C
ATOM	1418	N	MET A 162	55.794	3.147	28.443	1.00	22.46		N
ATOM	1419	CA	MET A 162	55.296	4.185	27.513	1.00	19.23		C
ATOM	1420	C	MET A 162	54.880	5.312	28.397	1.00	25.19		C
ATOM	1421	O	MET A 162	53.788	5.269	28.961	1.00	18.35		O
ATOM	1422	CB	MET A 162	53.979	3.796	26.850	1.00	15.55		C
ATOM	1423	CG	MET A 162	54.013	2.630	25.949	1.00	37.79		C
ATOM	1424	SD	MET A 162	54.354	3.100	24.235	1.00	52.07		S
ATOM	1425	CE	MET A 162	56.193	3.134	24.410	1.00	36.30		C
ATOM	1426	N	PRO A 163	55.730	6.313	28.521	1.00	18.43		N
ATOM	1427	CA	PRO A 163	55.390	7.472	29.337	1.00	17.76		C
ATOM	1428	C	PRO A 163	54.300	8.384	28.667	1.00	21.23		C
ATOM	1429	O	PRO A 163	54.208	8.448	27.433	1.00	15.20		O
ATOM	1430	CB	PRO A 163	56.727	8.196	29.423	1.00	11.43		C
ATOM	1431	CG	PRO A 163	57.352	7.874	28.031	1.00	13.99		C
ATOM	1432	CD	PRO A 163	57.086	6.401	27.949	1.00	12.24		C
ATOM	1433	N	THR A 164	53.478	9.060	29.478	1.00	13.95		N
ATOM	1434	CA	THR A 164	52.581	10.121	28.963	1.00	25.82		C
ATOM	1435	C	THR A 164	53.406	11.441	28.781	1.00	19.67		C
ATOM	1436	O	THR A 164	54.633	11.393	28.868	1.00	13.97		O
ATOM	1437	CB	THR A 164	51.373	10.391	29.903	1.00	25.51		C
ATOM	1438	OG1	THR A 164	50.470	11.321	29.267	1.00	14.77		O
ATOM	1439	CG2	THR A 164	51.818	10.886	31.298	1.00	9.06		C
ATOM	1440	N	ASN A 165	52.751	12.589	28.556	1.00	14.99		N
ATOM	1441	CA	ASN A 165	53.448	13.901	28.481	1.00	7.83		C
ATOM	1442	C	ASN A 165	54.167	14.064	29.824	1.00	11.21		C
ATOM	1443	O	ASN A 165	53.554	13.929	30.894	1.00	17.66		O
ATOM	1444	CB	ASN A 165	52.434	15.061	28.416	1.00	14.48		C
ATOM	1445	CG	ASN A 165	51.492	14.941	27.262	1.00	23.70		C
ATOM	1446	OD1	ASN A 165	51.939	14.800	26.129	1.00	22.37		O
ATOM	1447	ND2	ASN A 165	50.173	14.925	27.539	1.00	27.22		N
ATOM	1448	N	LEU A 166	55.418	14.490	29.777	1.00	8.23		N
ATOM	1449	CA	LEU A 166	56.187	14.604	30.994	1.00	14.40		C

ATOM	1450	C	LEU A 166	56.629	16.017	31.120	1.00	25.05	C
ATOM	1451	O	LEU A 166	56.624	16.718	30.125	1.00	25.09	O
ATOM	1452	CB	LEU A 166	57.460	13.743	30.870	1.00	17.48	C
ATOM	1453	CG	LEU A 166	57.423	12.218	30.652	1.00	16.63	C
ATOM	1454	CD1	LEU A 166	58.837	11.639	31.000	1.00	22.52	C
ATOM	1455	CD2	LEU A 166	56.336	11.539	31.514	1.00	7.46	C
ATOM	1456	N	TYR A 167	57.146	16.391	32.300	1.00	19.78	N
ATOM	1457	CA	TYR A 167	57.678	17.760	32.511	1.00	18.58	C
ATOM	1458	C	TYR A 167	58.534	17.763	33.767	1.00	15.53	C
ATOM	1459	O	TYR A 167	58.474	16.852	34.575	1.00	16.71	O
ATOM	1460	CB	TYR A 167	56.509	18.778	32.665	1.00	18.33	C
ATOM	1461	CG	TYR A 167	55.671	18.561	33.931	1.00	14.23	C
ATOM	1462	CD1	TYR A 167	54.624	17.618	33.977	1.00	13.35	C
ATOM	1463	CD2	TYR A 167	55.984	19.258	35.106	1.00	16.52	C
ATOM	1464	CE1	TYR A 167	53.889	17.446	35.146	1.00	21.17	C
ATOM	1465	CE2	TYR A 167	55.302	19.084	36.264	1.00	8.26	C
ATOM	1466	CZ	TYR A 167	54.228	18.203	36.296	1.00	23.56	C
ATOM	1467	OH	TYR A 167	53.526	18.078	37.504	1.00	22.81	O
ATOM	1468	N	GLY A 168	59.334	18.797	33.952	1.00	16.59	N
ATOM	1469	CA	GLY A 168	60.158	18.817	35.152	1.00	18.21	C
ATOM	1470	C	GLY A 168	61.534	19.428	34.880	1.00	13.69	C
ATOM	1471	O	GLY A 168	61.746	20.028	33.837	1.00	16.52	O
ATOM	1472	N	PRO A 169	62.473	19.263	35.817	1.00	20.33	N
ATOM	1473	CA	PRO A 169	63.801	19.822	35.656	1.00	16.07	C
ATOM	1474	C	PRO A 169	64.430	19.353	34.387	1.00	27.18	C
ATOM	1475	O	PRO A 169	64.305	18.186	33.981	1.00	21.23	O
ATOM	1476	CB	PRO A 169	64.595	19.206	36.805	1.00	17.28	C
ATOM	1477	CG	PRO A 169	63.649	18.919	37.830	1.00	19.89	C
ATOM	1478	CD	PRO A 169	62.263	18.772	37.189	1.00	22.47	C
ATOM	1479	N	HIS A 170	65.226	20.235	33.829	1.00	19.48	N
ATOM	1480	CA	HIS A 170	65.952	19.877	32.638	1.00	25.56	C
ATOM	1481	C	HIS A 170	65.096	19.707	31.428	1.00	29.15	C
ATOM	1482	O	HIS A 170	65.553	19.091	30.479	1.00	29.71	O
ATOM	1483	CB	HIS A 170	66.783	18.600	32.845	1.00	28.94	C
ATOM	1484	CG	HIS A 170	67.703	18.671	34.034	1.00	33.88	C
ATOM	1485	ND1	HIS A 170	68.975	19.203	33.969	1.00	25.46	N
ATOM	1486	CD2	HIS A 170	67.518	18.298	35.326	1.00	34.77	C
ATOM	1487	CE1	HIS A 170	69.531	19.151	35.166	1.00	25.63	C
ATOM	1488	NE2	HIS A 170	68.673	18.603	36.008	1.00	31.72	N
ATOM	1489	N	ASP A 171	63.881	20.245	31.440	1.00	21.52	N
ATOM	1490	CA	ASP A 171	63.041	20.267	30.218	1.00	28.63	C
ATOM	1491	C	ASP A 171	63.630	21.459	29.359	1.00	41.94	C
ATOM	1492	O	ASP A 171	64.534	22.171	29.835	1.00	29.69	O
ATOM	1493	CB	ASP A 171	61.552	20.558	30.602	1.00	26.40	C
ATOM	1494	CG	ASP A 171	60.552	20.097	29.540	1.00	22.32	C

ATOM	1495	OD1	ASP	A	171	60.890	20.067	28.325	1.00 32.03	O
ATOM	1496	OD2	ASP	A	171	59.427	19.719	29.916	1.00 42.13	O
ATOM	1497	N	ASN	A	172	63.141	21.712	28.137	1.00 42.08	N
ATOM	1498	CA	ASN	A	172	63.616	22.893	27.388	1.00 35.95	C
ATOM	1499	C	ASN	A	172	62.665	24.056	27.674	1.00 33.71	C
ATOM	1500	O	ASN	A	172	61.586	24.102	27.104	1.00 32.69	O
ATOM	1501	CB	ASN	A	172	63.632	22.667	25.869	1.00 41.60	C
ATOM	1502	CG	ASN	A	172	63.807	23.987	25.086	1.00 39.09	C
ATOM	1503	OD1	ASN	A	172	62.973	24.347	24.259	1.00 83.94	O
ATOM	1504	ND2	ASN	A	172	64.855	24.740	25.418	1.00 65.07	N
ATOM	1505	N	PHE	A	173	63.021	24.953	28.583	1.00 31.93	N
ATOM	1506	CA	PHE	A	173	62.082	26.030	28.944	1.00 48.24	C
ATOM	1507	C	PHE	A	173	61.989	27.260	28.045	1.00 69.01	C
ATOM	1508	O	PHE	A	173	62.278	28.395	28.465	1.00 58.79	O
ATOM	1509	CB	PHE	A	173	62.225	26.459	30.390	1.00 43.43	C
ATOM	1510	CG	PHE	A	173	61.867	25.399	31.356	1.00 34.19	C
ATOM	1511	CD1	PHE	A	173	62.810	24.488	31.751	1.00 24.68	C
ATOM	1512	CD2	PHE	A	173	60.621	25.354	31.925	1.00 24.84	C
ATOM	1513	CE1	PHE	A	173	62.524	23.548	32.682	1.00 23.64	C
ATOM	1514	CE2	PHE	A	173	60.305	24.366	32.804	1.00 31.32	C
ATOM	1515	CZ	PHE	A	173	61.263	23.457	33.192	1.00 24.30	C
ATOM	1516	N	HIS	A	174	61.510	27.036	26.831	1.00 68.16	N
ATOM	1517	CA	HIS	A	174	61.401	28.109	25.871	1.00 64.53	C
ATOM	1518	C	HIS	A	174	59.973	28.221	25.400	1.00 71.58	C
ATOM	1519	O	HIS	A	174	59.309	27.186	25.249	1.00 73.20	O
ATOM	1520	CB	HIS	A	174	62.418	27.870	24.736	1.00 71.71	C
ATOM	1521	CG	HIS	A	174	63.835	27.868	25.229	1.00 92.29	C
ATOM	1522	ND1	HIS	A	174	64.921	27.539	24.440	1.00100.00	N
ATOM	1523	CD2	HIS	A	174	64.338	28.133	26.463	1.00100.00	C
ATOM	1524	CE1	HIS	A	174	66.032	27.628	25.160	1.00100.00	C
ATOM	1525	NE2	HIS	A	174	65.705	27.981	26.393	1.00100.00	N
ATOM	1526	N	PRO	A	175	59.469	29.461	25.262	1.00 65.71	N
ATOM	1527	CA	PRO	A	175	58.109	29.658	24.770	1.00 55.72	C
ATOM	1528	C	PRO	A	175	58.233	29.297	23.267	1.00 75.83	C
ATOM	1529	O	PRO	A	175	57.224	29.226	22.554	1.00 69.59	O
ATOM	1530	CB	PRO	A	175	57.866	31.142	25.026	1.00 49.14	C
ATOM	1531	CG	PRO	A	175	59.258	31.790	24.901	1.00 42.23	C
ATOM	1532	CD	PRO	A	175	60.286	30.695	25.109	1.00 49.59	C
ATOM	1533	N	SER	A	176	59.480	28.954	22.879	1.00 85.09	N
ATOM	1534	CA	SER	A	176	59.954	28.474	21.548	1.00 81.18	C
ATOM	1535	C	SER	A	176	59.660	26.965	21.343	1.00 73.90	C
ATOM	1536	O	SER	A	176	59.617	26.458	20.213	1.00 57.03	O
ATOM	1537	CB	SER	A	176	61.493	28.666	21.447	1.00 71.32	C
ATOM	1538	OG	SER	A	176	62.048	29.349	22.578	1.00 51.93	O
ATOM	1539	N	ASN	A	177	59.520	26.276	22.480	1.00 66.23	N

ATOM 1540 CA ASN A 177 59.274 24.847 22.619 1.00 56.41 C
ATOM 1541 C ASN A 177 57.810 24.497 22.353 1.00 60.91 C
ATOM 1542 O ASN A 177 56.914 25.215 22.811 1.00 55.58 O
ATOM 1543 CB ASN A 177 59.619 24.469 24.065 1.00 50.45 C
ATOM 1544 CG ASN A 177 59.562 22.970 24.319 1.00 66.57 C
ATOM 1545 OD1 ASN A 177 59.095 22.216 23.476 1.00100.00 O
ATOM 1546 ND2 ASN A 177 60.099 22.546 25.464 1.00 35.61 N
ATOM 1547 N SER A 178 57.583 23.387 21.627 1.00 57.10 N
ATOM 1548 CA SER A 178 56.234 22.853 21.279 1.00 50.50 C
ATOM 1549 C SER A 178 55.557 22.159 22.491 1.00 76.24 C
ATOM 1550 O SER A 178 54.575 21.400 22.304 1.00 99.63 O
ATOM 1551 CB SER A 178 56.316 21.800 20.118 1.00 10.17 C
ATOM 1552 OG SER A 178 57.397 22.112 19.217 1.00 71.69 O
ATOM 1553 N HIS A 179 56.134 22.284 23.694 1.00 37.39 N
ATOM 1554 CA HIS A 179 55.569 21.587 24.855 1.00 30.96 C
ATOM 1555 C HIS A 179 54.961 22.616 25.767 1.00 21.93 C
ATOM 1556 O HIS A 179 55.641 23.598 26.138 1.00 25.17 O
ATOM 1557 CB HIS A 179 56.634 20.683 25.575 1.00 36.20 C
ATOM 1558 CG HIS A 179 56.973 19.419 24.835 1.00 42.90 C
ATOM 1559 ND1 HIS A 179 56.973 19.335 23.457 1.00 49.52 N
ATOM 1560 CD2 HIS A 179 57.323 18.190 25.278 1.00 52.42 C
ATOM 1561 CE1 HIS A 179 57.283 18.109 23.084 1.00 44.78 C
ATOM 1562 NE2 HIS A 179 57.500 17.393 24.168 1.00 50.49 N
ATOM 1563 N VAL A 180 53.661 22.454 26.038 1.00 19.14 N
ATOM 1564 CA VAL A 180 52.886 23.449 26.789 1.00 29.03 C
ATOM 1565 C VAL A 180 53.373 23.890 28.142 1.00 31.29 C
ATOM 1566 O VAL A 180 53.348 25.075 28.447 1.00 19.55 O
ATOM 1567 CB VAL A 180 51.403 23.115 26.914 1.00 35.47 C
ATOM 1568 CG1 VAL A 180 50.630 24.399 27.217 1.00 35.84 C
ATOM 1569 CG2 VAL A 180 50.923 22.550 25.663 1.00 36.11 C
ATOM 1570 N ILE A 181 53.684 22.935 29.005 1.00 26.57 N
ATOM 1571 CA ILE A 181 54.138 23.285 30.360 1.00 24.49 C
ATOM 1572 C ILE A 181 55.371 24.213 30.361 1.00 16.51 C
ATOM 1573 O ILE A 181 55.326 25.315 30.909 1.00 24.42 O
ATOM 1574 CB ILE A 181 54.285 22.018 31.264 1.00 20.20 C
ATOM 1575 CG1 ILE A 181 52.878 21.428 31.528 1.00 18.22 C
ATOM 1576 CG2 ILE A 181 55.014 22.315 32.581 1.00 13.37 C
ATOM 1577 CD1 ILE A 181 52.867 20.086 32.286 1.00 8.03 C
ATOM 1578 N PRO A 182 56.452 23.779 29.718 1.00 22.21 N
ATOM 1579 CA PRO A 182 57.664 24.605 29.640 1.00 22.07 C
ATOM 1580 C PRO A 182 57.379 25.852 28.828 1.00 24.18 C
ATOM 1581 O PRO A 182 57.811 26.949 29.210 1.00 18.35 O
ATOM 1582 CB PRO A 182 58.682 23.725 28.890 1.00 24.97 C
ATOM 1583 CG PRO A 182 57.925 22.473 28.471 1.00 25.77 C
ATOM 1584 CD PRO A 182 56.727 22.359 29.401 1.00 18.23 C

ATOM	1585	N	ALA A 183	56.628	25.707	27.729	1.00 21.45		N
ATOM	1586	CA	ALA A 183	56.261	26.896	26.943	1.00 21.66		C
ATOM	1587	C	ALA A 183	55.464	27.900	27.811	1.00 26.10		C
ATOM	1588	O	ALA A 183	55.773	29.091	27.856	1.00 19.50		O
ATOM	1589	CB	ALA A 183	55.473	26.513	25.703	1.00 13.26		C
ATOM	1590	N	LEU A 184	54.472	27.389	28.543	1.00 23.34		N
ATOM	1591	CA	LEU A 184	53.642	28.215	29.401	1.00 19.05		C
ATOM	1592	C	LEU A 184	54.312	28.693	30.655	1.00 21.91		C
ATOM	1593	O	LEU A 184	54.017	29.771	31.158	1.00 19.71		O
ATOM	1594	CB	LEU A 184	52.309	27.553	29.715	1.00 14.41		C
ATOM	1595	CG	LEU A 184	51.342	27.595	28.525	1.00 23.42		C
ATOM	1596	CD1	LEU A 184	49.918	27.244	28.928	1.00 31.06		C
ATOM	1597	CD2	LEU A 184	51.380	28.896	27.690	1.00 21.73		C
ATOM	1598	N	LEU A 185	55.178	27.879	31.213	1.00 18.39		N
ATOM	1599	CA	LEU A 185	55.833	28.332	32.417	1.00 16.39		C
ATOM	1600	C	LEU A 185	56.669	29.528	31.985	1.00 23.67		C
ATOM	1601	O	LEU A 185	56.681	30.590	32.644	1.00 29.38		O
ATOM	1602	CB	LEU A 185	56.723	27.233	33.015	1.00 15.05		C
ATOM	1603	CG	LEU A 185	56.021	26.348	34.041	1.00 15.56		C
ATOM	1604	CD1	LEU A 185	56.819	25.022	34.301	1.00 21.06		C
ATOM	1605	CD2	LEU A 185	55.722	27.113	35.321	1.00 11.02		C
ATOM	1606	N	ARG A 186	57.337	29.397	30.852	1.00 17.09		N
ATOM	1607	CA	ARG A 186	58.137	30.523	30.429	1.00 18.82		C
ATOM	1608	C	ARG A 186	57.308	31.752	30.069	1.00 29.00		C
ATOM	1609	O	ARG A 186	57.629	32.880	30.476	1.00 23.91		O
ATOM	1610	CB	ARG A 186	59.026	30.146	29.281	1.00 22.06		C
ATOM	1611	CG	ARG A 186	59.653	31.365	28.652	1.00 38.46		C
ATOM	1612	CD	ARG A 186	60.825	31.804	29.462	1.00 83.66		C
ATOM	1613	NE	ARG A 186	62.012	31.861	28.631	1.00 70.77		N
ATOM	1614	CZ	ARG A 186	63.058	32.622	28.904	1.00 91.68		C
ATOM	1615	NH1	ARG A 186	63.053	33.386	29.995	1.00 56.56		N
ATOM	1616	NH2	ARG A 186	64.098	32.639	28.082	1.00100.00		N
ATOM	1617	N	ARG A 187	56.234	31.544	29.310	1.00 20.96		N
ATOM	1618	CA	ARG A 187	55.361	32.662	28.941	1.00 19.32		C
ATOM	1619	C	ARG A 187	54.765	33.453	30.142	1.00 28.41		C
ATOM	1620	O	ARG A 187	54.823	34.700	30.193	1.00 17.23		O
ATOM	1621	CB	ARG A 187	54.270	32.223	27.957	1.00 17.05		C
ATOM	1622	CG	ARG A 187	54.813	31.546	26.720	1.00 61.42		C
ATOM	1623	CD	ARG A 187	53.696	31.244	25.757	1.00 44.57		C
ATOM	1624	NE	ARG A 187	53.033	32.472	25.354	1.00 29.47		N
ATOM	1625	CZ	ARG A 187	51.831	32.534	24.790	1.00 17.82		C
ATOM	1626	NH1	ARG A 187	51.136	31.427	24.544	1.00 24.95		N
ATOM	1627	NH2	ARG A 187	51.341	33.716	24.447	1.00 37.77		N
ATOM	1628	N	PHE A 188	54.192	32.734	31.101	1.00 23.48		N
ATOM	1629	CA	PHE A 188	53.604	33.399	32.259	1.00 21.24		C

ATOM 1630 C PHE A 188 54.638 34.080 33.095 1.00 21.39 C
ATOM 1631 O PHE A 188 54.394 35.126 33.626 1.00 23.90 O
ATOM 1632 CB PHE A 188 52.723 32.466 33.077 1.00 19.95 C
ATOM 1633 CG PHE A 188 51.389 32.215 32.435 1.00 22.28 C
ATOM 1634 CD1 PHE A 188 50.440 33.229 32.375 1.00 19.42 C
ATOM 1635 CD2 PHE A 188 51.144 31.038 31.734 1.00 23.82 C
ATOM 1636 CE1 PHE A 188 49.191 33.026 31.742 1.00 24.77 C
ATOM 1637 CE2 PHE A 188 49.936 30.826 31.057 1.00 20.17 C
ATOM 1638 CZ PHE A 188 48.945 31.815 31.068 1.00 23.14 C
ATOM 1639 N HIS A 189 55.831 33.513 33.118 1.00 24.15 N
ATOM 1640 CA HIS A 189 56.933 34.122 33.837 1.00 28.79 C
ATOM 1641 C HIS A 189 57.303 35.506 33.315 1.00 28.58 C
ATOM 1642 O HIS A 189 57.480 36.463 34.083 1.00 20.07 O
ATOM 1643 CB HIS A 189 58.148 33.268 33.641 1.00 31.38 C
ATOM 1644 CG HIS A 189 59.364 33.844 34.290 1.00 29.98 C
ATOM 1645 ND1 HIS A 189 59.548 33.833 35.658 1.00 31.00 N
ATOM 1646 CD2 HIS A 189 60.449 34.464 33.766 1.00 21.79 C
ATOM 1647 CE1 HIS A 189 60.722 34.371 35.945 1.00 24.04 C
ATOM 1648 NE2 HIS A 189 61.257 34.815 34.821 1.00 19.53 N
ATOM 1649 N GLU A 190 57.539 35.561 32.006 1.00 28.43 N
ATOM 1650 CA GLU A 190 57.876 36.816 31.324 1.00 27.72 C
ATOM 1651 C GLU A 190 56.725 37.829 31.437 1.00 32.56 C
ATOM 1652 O GLU A 190 56.949 38.995 31.717 1.00 27.06 O
ATOM 1653 CB GLU A 190 58.122 36.529 29.849 1.00 28.55 C
ATOM 1654 CG GLU A 190 59.150 35.461 29.614 1.00 35.29 C
ATOM 1655 CD GLU A 190 60.553 35.941 29.892 1.00 99.81 C
ATOM 1656 OE1 GLU A 190 60.913 36.037 31.085 1.00 86.56 O
ATOM 1657 OE2 GLU A 190 61.293 36.167 28.910 1.00100.00 O
ATOM 1658 N ALA A 191 55.493 37.391 31.196 1.00 32.67 N
ATOM 1659 CA ALA A 191 54.349 38.286 31.311 1.00 25.30 C
ATOM 1660 C ALA A 191 54.287 38.795 32.742 1.00 36.20 C
ATOM 1661 O ALA A 191 53.920 39.924 33.014 1.00 27.52 O
ATOM 1662 CB ALA A 191 53.055 37.563 31.000 1.00 16.48 C
ATOM 1663 N THR A 192 54.549 37.927 33.693 1.00 29.39 N
ATOM 1664 CA THR A 192 54.395 38.386 35.041 1.00 19.08 C
ATOM 1665 C THR A 192 55.420 39.494 35.298 1.00 44.78 C
ATOM 1666 O THR A 192 55.094 40.550 35.839 1.00 40.58 O
ATOM 1667 CB THR A 192 54.515 37.235 35.983 1.00 18.99 C
ATOM 1668 OG1 THR A 192 53.410 36.348 35.755 1.00 34.36 O
ATOM 1669 CG2 THR A 192 54.461 37.738 37.425 1.00 21.15 C
ATOM 1670 N ALA A 193 56.617 39.312 34.757 1.00 48.58 N
ATOM 1671 CA ALA A 193 57.705 40.286 34.905 1.00 50.59 C
ATOM 1672 C ALA A 193 57.496 41.613 34.145 1.00 54.42 C
ATOM 1673 O ALA A 193 57.952 42.698 34.553 1.00 48.28 O
ATOM 1674 CB ALA A 193 59.047 39.640 34.496 1.00 51.78 C

ATOM	1675	N	GLN A 194	56.810	41.530	33.022	1.00 43.16		N
ATOM	1676	CA	GLN A 194	56.586	42.722	32.242	1.00 38.03		C
ATOM	1677	C	GLN A 194	55.264	43.389	32.576	1.00 40.85		C
ATOM	1678	O	GLN A 194	54.830	44.284	31.845	1.00 51.20		O
ATOM	1679	CB	GLN A 194	56.599	42.358	30.750	1.00 35.96		C
ATOM	1680	CG	GLN A 194	57.910	41.692	30.290	1.00100.00		C
ATOM	1681	CD	GLN A 194	57.715	40.661	29.158	1.00100.00		C
ATOM	1682	OE1	GLN A 194	56.619	40.546	28.579	1.00100.00		O
ATOM	1683	NE2	GLN A 194	58.782	39.904	28.848	1.00100.00		N
ATOM	1684	N	GLY A 195	54.583	42.949	33.630	1.00 32.29		N
ATOM	1685	CA	GLY A 195	53.236	43.464	33.864	1.00 36.26		C
ATOM	1686	C	GLY A 195	52.299	43.332	32.593	1.00 45.33		C
ATOM	1687	O	GLY A 195	51.515	44.242	32.346	1.00 45.16		O
ATOM	1688	N	GLY A 196	52.405	42.245	31.788	1.00 36.33		N
ATOM	1689	CA	GLY A 196	51.515	41.965	30.608	1.00 19.06		C
ATOM	1690	C	GLY A 196	50.037	41.958	31.117	1.00 22.49		C
ATOM	1691	O	GLY A 196	49.724	41.479	32.223	1.00 33.09		O
ATOM	1692	N	PRO A 197	49.144	42.657	30.431	1.00 29.22		N
ATOM	1693	CA	PRO A 197	47.790	42.732	30.953	1.00 25.29		C
ATOM	1694	C	PRO A 197	47.091	41.413	30.674	1.00 24.64		C
ATOM	1695	O	PRO A 197	46.192	40.991	31.411	1.00 24.75		O
ATOM	1696	CB	PRO A 197	47.162	43.911	30.176	1.00 26.31		C
ATOM	1697	CG	PRO A 197	48.188	44.407	29.252	1.00 26.56		C
ATOM	1698	CD	PRO A 197	49.307	43.454	29.203	1.00 30.25		C
ATOM	1699	N	ASP A 198	47.572	40.723	29.658	1.00 16.88		N
ATOM	1700	CA	ASP A 198	47.067	39.418	29.405	1.00 21.65		C
ATOM	1701	C	ASP A 198	48.046	38.522	28.677	1.00 31.28		C
ATOM	1702	O	ASP A 198	49.062	38.978	28.172	1.00 34.57		O
ATOM	1703	CB	ASP A 198	45.739	39.507	28.669	1.00 32.80		C
ATOM	1704	CG	ASP A 198	45.868	40.055	27.256	1.00 46.13		C
ATOM	1705	OD1	ASP A 198	46.982	40.230	26.725	1.00 57.45		O
ATOM	1706	OD2	ASP A 198	44.817	40.271	26.640	1.00 67.61		O
ATOM	1707	N	VAL A 199	47.713	37.234	28.614	1.00 38.67		N
ATOM	1708	CA	VAL A 199	48.499	36.226	27.901	1.00 27.79		C
ATOM	1709	C	VAL A 199	47.462	35.469	27.065	1.00 25.88		C
ATOM	1710	O	VAL A 199	46.460	35.023	27.598	1.00 24.22		O
ATOM	1711	CB	VAL A 199	49.163	35.229	28.905	1.00 24.37		C
ATOM	1712	CG1	VAL A 199	49.874	34.047	28.160	1.00 20.28		C
ATOM	1713	CG2	VAL A 199	50.121	35.942	29.835	1.00 22.25		C
ATOM	1714	N	VAL A 200	47.661	35.386	25.757	1.00 23.72		N
ATOM	1715	CA	VAL A 200	46.701	34.694	24.903	1.00 23.99		C
ATOM	1716	C	VAL A 200	47.167	33.286	24.499	1.00 22.85		C
ATOM	1717	O	VAL A 200	48.321	33.108	24.188	1.00 29.77		O
ATOM	1718	CB	VAL A 200	46.358	35.548	23.680	1.00 23.11		C
ATOM	1719	CG1	VAL A 200	45.561	34.737	22.598	1.00 16.25		C

ATOM 1720 CG2 VAL A 200 45.652 36.823 24.130 1.00 27.86 C
ATOM 1721 N VAL A 201 46.296 32.278 24.632 1.00 27.39 N
ATOM 1722 CA VAL A 201 46.588 30.893 24.265 1.00 9.63 C
ATOM 1723 C VAL A 201 45.653 30.529 23.165 1.00 19.63 C
ATOM 1724 O VAL A 201 44.452 30.755 23.312 1.00 17.61 O
ATOM 1725 CB VAL A 201 46.306 29.952 25.426 1.00 19.95 C
ATOM 1726 CG1 VAL A 201 46.703 28.519 25.054 1.00 20.85 C
ATOM 1727 CG2 VAL A 201 47.086 30.439 26.661 1.00 16.73 C
ATOM 1728 N TRP A 202 46.210 30.080 22.030 1.00 14.36 N
ATOM 1729 CA TRP A 202 45.422 29.693 20.865 1.00 18.97 C
ATOM 1730 C TRP A 202 44.495 28.572 21.313 1.00 36.22 C
ATOM 1731 O TRP A 202 44.934 27.694 22.057 1.00 31.46 O
ATOM 1732 CB TRP A 202 46.292 29.055 19.823 1.00 19.14 C
ATOM 1733 CG TRP A 202 47.243 29.894 19.066 1.00 33.65 C
ATOM 1734 CD1 TRP A 202 48.391 29.463 18.429 1.00 35.28 C
ATOM 1735 CD2 TRP A 202 47.126 31.282 18.772 1.00 39.90 C
ATOM 1736 NE1 TRP A 202 48.941 30.481 17.693 1.00 37.86 N
ATOM 1737 CE2 TRP A 202 48.228 31.624 17.922 1.00 38.35 C
ATOM 1738 CE3 TRP A 202 46.206 32.281 19.138 1.00 39.39 C
ATOM 1739 CZ2 TRP A 202 48.380 32.884 17.367 1.00 36.15 C
ATOM 1740 CZ3 TRP A 202 46.356 33.542 18.578 1.00 39.60 C
ATOM 1741 CH2 TRP A 202 47.428 33.828 17.684 1.00 40.99 C
ATOM 1742 N GLY A 203 43.245 28.564 20.842 1.00 25.59 N
ATOM 1743 CA GLY A 203 42.332 27.483 21.169 1.00 13.09 C
ATOM 1744 C GLY A 203 41.260 27.813 22.193 1.00 21.12 C
ATOM 1745 O GLY A 203 41.340 28.815 22.886 1.00 22.86 O
ATOM 1746 N SER A 204 40.270 26.919 22.262 1.00 16.88 N
ATOM 1747 CA SER A 204 39.163 26.979 23.192 1.00 18.36 C
ATOM 1748 C SER A 204 39.561 26.664 24.659 1.00 22.07 C
ATOM 1749 O SER A 204 38.888 27.096 25.604 1.00 34.39 O
ATOM 1750 CB SER A 204 38.053 25.998 22.740 1.00 9.99 C
ATOM 1751 OG SER A 204 38.237 24.695 23.291 1.00 16.37 O
ATOM 1752 N GLY A 205 40.562 25.813 24.854 1.00 12.42 N
ATOM 1753 CA GLY A 205 40.963 25.411 26.208 1.00 11.64 C
ATOM 1754 C GLY A 205 40.208 24.178 26.711 1.00 19.49 C
ATOM 1755 O GLY A 205 40.422 23.723 27.838 1.00 13.59 O
ATOM 1756 N THR A 206 39.292 23.683 25.881 1.00 15.38 N
ATOM 1757 CA THR A 206 38.432 22.594 26.281 1.00 10.80 C
ATOM 1758 C THR A 206 39.056 21.221 26.154 1.00 26.39 C
ATOM 1759 O THR A 206 38.564 20.267 26.737 1.00 23.28 O
ATOM 1760 CB THR A 206 37.124 22.562 25.460 1.00 12.86 C
ATOM 1761 OG1 THR A 206 37.438 22.395 24.082 1.00 13.12 O
ATOM 1762 CG2 THR A 206 36.348 23.840 25.620 1.00 10.62 C
ATOM 1763 N PRO A 207 40.101 21.083 25.354 1.00 21.10 N
ATOM 1764 CA PRO A 207 40.658 19.743 25.175 1.00 18.15 C

ATOM 1765 C PRO A 207 41.316 19.181 26.423 1.00 21.75 C
ATOM 1766 O PRO A 207 41.951 19.925 27.215 1.00 20.65 O
ATOM 1767 CB PRO A 207 41.638 19.909 24.013 1.00 17.51 C
ATOM 1768 CG PRO A 207 41.146 21.213 23.307 1.00 21.45 C
ATOM 1769 CD PRO A 207 40.698 22.062 24.431 1.00 23.44 C
ATOM 1770 N MET A 208 41.112 17.876 26.624 1.00 15.60 N
ATOM 1771 CA MET A 208 41.694 17.167 27.775 1.00 22.94 C
ATOM 1772 C MET A 208 43.058 16.427 27.579 1.00 21.90 C
ATOM 1773 O MET A 208 43.248 15.677 26.633 1.00 23.16 O
ATOM 1774 CB MET A 208 40.645 16.273 28.386 1.00 32.86 C
ATOM 1775 CG MET A 208 39.630 17.057 29.223 1.00 46.17 C
ATOM 1776 SD MET A 208 38.301 15.990 29.826 1.00 57.85 S
ATOM 1777 CE MET A 208 37.999 15.028 28.343 1.00 58.23 C
ATOM 1778 N ARG A 209 44.022 16.681 28.456 1.00 17.75 N
ATOM 1779 CA ARG A 209 45.318 16.042 28.324 1.00 19.88 C
ATOM 1780 C ARG A 209 45.871 15.534 29.639 1.00 16.92 C
ATOM 1781 O ARG A 209 45.433 15.946 30.697 1.00 16.58 O
ATOM 1782 CB ARG A 209 46.340 16.963 27.658 1.00 21.07 C
ATOM 1783 CG ARG A 209 45.980 17.478 26.275 1.00 22.57 C
ATOM 1784 CD ARG A 209 45.833 16.357 25.282 1.00 28.26 C
ATOM 1785 NE ARG A 209 45.586 16.819 23.906 1.00 23.15 N
ATOM 1786 CZ ARG A 209 44.420 16.742 23.267 1.00 34.52 C
ATOM 1787 NH1 ARG A 209 43.336 16.267 23.890 1.00 18.03 N
ATOM 1788 NH2 ARG A 209 44.339 17.175 22.012 1.00 29.78 N
ATOM 1789 N GLU A 210 46.878 14.675 29.547 1.00 20.87 N
ATOM 1790 CA GLU A 210 47.530 14.079 30.720 1.00 17.37 C
ATOM 1791 C GLU A 210 49.031 14.490 30.851 1.00 20.96 C
ATOM 1792 O GLU A 210 49.748 14.622 29.841 1.00 22.44 O
ATOM 1793 CB GLU A 210 47.400 12.562 30.571 1.00 16.26 C
ATOM 1794 CG GLU A 210 47.807 11.785 31.809 1.00 19.91 C
ATOM 1795 CD GLU A 210 48.057 10.304 31.531 1.00 27.81 C
ATOM 1796 OE1 GLU A 210 48.111 9.919 30.343 1.00 17.29 O
ATOM 1797 OE2 GLU A 210 48.268 9.540 32.494 1.00 21.63 O
ATOM 1798 N PHE A 211 49.504 14.712 32.084 1.00 14.02 N
ATOM 1799 CA PHE A 211 50.887 15.159 32.353 1.00 17.48 C
ATOM 1800 C PHE A 211 51.458 14.414 33.531 1.00 33.62 C
ATOM 1801 O PHE A 211 50.716 14.031 34.443 1.00 27.96 O
ATOM 1802 CB PHE A 211 50.933 16.677 32.644 1.00 17.78 C
ATOM 1803 CG PHE A 211 50.303 17.490 31.541 1.00 21.49 C
ATOM 1804 CD1 PHE A 211 51.009 17.676 30.320 1.00 17.36 C
ATOM 1805 CD2 PHE A 211 48.933 17.844 31.618 1.00 15.09 C
ATOM 1806 CE1 PHE A 211 50.399 18.334 29.237 1.00 16.37 C
ATOM 1807 CE2 PHE A 211 48.288 18.491 30.533 1.00 9.61 C
ATOM 1808 CZ PHE A 211 49.053 18.756 29.344 1.00 12.71 C
ATOM 1809 N LEU A 212 52.761 14.161 33.495 1.00 23.76 N

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ATOM   1810  CA  LEU A 212      53.405  13.448  34.603  1.00 21.24           C
ATOM   1811  C   LEU A 212      54.772  14.053  34.898  1.00 14.00           C
ATOM   1812  O   LEU A 212      55.519  14.398  33.985  1.00 13.99           O
ATOM   1813  CB  LEU A 212      53.548  11.954  34.294  1.00 21.52           C
ATOM   1814  CG  LEU A 212      54.033  11.039  35.406  1.00 21.09           C
ATOM   1815  CD1 LEU A 212      52.866  10.634  36.280  1.00 20.84           C
ATOM   1816  CD2 LEU A 212      54.768   9.829  34.832  1.00 13.18           C
ATOM   1817  N   HIS A 213      55.023  14.302  36.175  1.00  9.60           N
ATOM   1818  CA  HIS A 213      56.290  14.864  36.555  1.00 13.66           C
ATOM   1819  C   HIS A 213      57.380  13.828  36.293  1.00 20.37           C
ATOM   1820  O   HIS A 213      57.238  12.614  36.542  1.00 16.08           O
ATOM   1821  CB  HIS A 213      56.280  15.250  38.002  1.00 18.72           C
ATOM   1822  CG  HIS A 213      57.491  16.017  38.408  1.00 21.22           C
ATOM   1823  ND1 HIS A 213      58.703  15.406  38.656  1.00 24.29           N
ATOM   1824  CD2 HIS A 213      57.716  17.353  38.499  1.00 23.67           C
ATOM   1825  CE1 HIS A 213      59.615  16.331  38.917  1.00 19.13           C
ATOM   1826  NE2 HIS A 213      59.041  17.523  38.847  1.00 21.99           N
ATOM   1827  N   VAL A 214      58.459  14.295  35.698  1.00 21.07           N
ATOM   1828  CA  VAL A 214      59.532  13.383  35.361  1.00 19.23           C
ATOM   1829  C   VAL A 214      60.067  12.523  36.551  1.00 27.20           C
ATOM   1830  O   VAL A 214      60.604  11.444  36.359  1.00 22.23           O
ATOM   1831  CB  VAL A 214      60.625  14.125  34.566  1.00 11.84           C
ATOM   1832  CG1 VAL A 214      61.390  15.199  35.485  1.00  8.52           C
ATOM   1833  CG2 VAL A 214      61.560  13.097  33.902  1.00 12.39           C
ATOM   1834  N   ASP A 215      59.893  12.984  37.790  1.00 25.29           N
ATOM   1835  CA  ASP A 215      60.406  12.228  38.936  1.00 18.19           C
ATOM   1836  C   ASP A 215      59.530  11.023  39.230  1.00 13.85           C
ATOM   1837  O   ASP A 215      59.988   9.981  39.666  1.00 17.44           O
ATOM   1838  CB  ASP A 215      60.575  13.129  40.155  1.00 16.27           C
ATOM   1839  CG  ASP A 215      61.859  13.979  40.068  1.00 30.73           C
ATOM   1840  OD1 ASP A 215      62.782  13.614  39.308  1.00 23.02           O
ATOM   1841  OD2 ASP A 215      61.957  15.029  40.730  1.00 26.00           O
ATOM   1842  N   ASP A 216      58.276  11.136  38.863  1.00 20.08           N
ATOM   1843  CA  ASP A 216      57.378  10.017  39.016  1.00 18.78           C
ATOM   1844  C   ASP A 216      57.761   9.083  37.894  1.00 23.56           C
ATOM   1845  O   ASP A 216      57.715   7.880  38.026  1.00 20.79           O
ATOM   1846  CB  ASP A 216      55.912  10.457  38.821  1.00 17.18           C
ATOM   1847  CG  ASP A 216      55.193  10.757  40.162  1.00 38.03           C
ATOM   1848  OD1 ASP A 216      55.503  10.119  41.223  1.00 26.02           O
ATOM   1849  OD2 ASP A 216      54.249  11.587  40.124  1.00 25.41           O
ATOM   1850  N   MET A 217      58.092   9.653  36.755  1.00 18.11           N
ATOM   1851  CA  MET A 217      58.394   8.785  35.636  1.00 22.41           C
ATOM   1852  C   MET A 217      59.572   7.942  35.992  1.00 27.54           C
ATOM   1853  O   MET A 217      59.579   6.752  35.710  1.00 20.86           O
ATOM   1854  CB  MET A 217      58.637   9.592  34.345  1.00 21.24           C
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ATOM   1855  CG  MET A 217      59.478   8.918  33.287  1.00 16.37           C
ATOM   1856  SD  MET A 217      58.962   7.412  32.473  1.00 30.51           S
ATOM   1857  CE  MET A 217      57.465   7.608  32.391  1.00 19.57           C
ATOM   1858  N   ALA A 218      60.561   8.562  36.623  1.00 19.09           N
ATOM   1859  CA  ALA A 218      61.774   7.841  37.002  1.00 13.65           C
ATOM   1860  C   ALA A 218      61.436   6.778  38.028  1.00 22.61           C
ATOM   1861  O   ALA A 218      61.934   5.670  37.967  1.00 19.36           O
ATOM   1862  CB  ALA A 218      62.809   8.780  37.579  1.00 12.23           C
ATOM   1863  N   ALA A 219      60.605   7.109  39.000  1.00 19.34           N
ATOM   1864  CA  ALA A 219      60.310   6.105  40.023  1.00 18.01           C
ATOM   1865  C   ALA A 219      59.630   4.901  39.413  1.00 23.57           C
ATOM   1866  O   ALA A 219      59.781   3.777  39.898  1.00 22.71           O
ATOM   1867  CB  ALA A 219      59.387   6.678  41.083  1.00 10.11           C
ATOM   1868  N   ALA A 220      58.753   5.174  38.454  1.00 18.99           N
ATOM   1869  CA  ALA A 220      57.905   4.158  37.855  1.00 14.12           C
ATOM   1870  C   ALA A 220      58.753   3.213  37.034  1.00 25.33           C
ATOM   1871  O   ALA A 220      58.584   2.006  37.114  1.00 20.63           O
ATOM   1872  CB  ALA A 220      56.796   4.798  37.023  1.00  8.53           C
ATOM   1873  N   SER A 221      59.770   3.772  36.379  1.00 23.92           N
ATOM   1874  CA  SER A 221      60.702   3.011  35.556  1.00 18.38           C
ATOM   1875  C   SER A 221      61.537   1.989  36.353  1.00 20.90           C
ATOM   1876  O   SER A 221      61.683   0.799  35.983  1.00 19.84           O
ATOM   1877  CB  SER A 221      61.604   3.985  34.804  1.00 10.67           C
ATOM   1878  OG  SER A 221      60.847   4.744  33.867  1.00 15.61           O
ATOM   1879  N   ILE A 222      62.083   2.476  37.463  1.00 18.12           N
ATOM   1880  CA  ILE A 222      62.866   1.644  38.381  1.00 21.56           C
ATOM   1881  C   ILE A 222      62.020   0.554  39.068  1.00 29.10           C
ATOM   1882  O   ILE A 222      62.504  -0.566  39.307  1.00 19.03           O
ATOM   1883  CB  ILE A 222      63.467   2.516  39.432  1.00 24.56           C
ATOM   1884  CG1 ILE A 222      64.465   3.473  38.765  1.00 32.13           C
ATOM   1885  CG2 ILE A 222      64.129   1.671  40.500  1.00 28.26           C
ATOM   1886  CD1 ILE A 222      64.973   4.585  39.649  1.00 15.61           C
ATOM   1887  N   HIS A 223      60.772   0.907  39.384  1.00 19.34           N
ATOM   1888  CA  HIS A 223      59.829  -0.031  39.996  1.00 20.46           C
ATOM   1889  C   HIS A 223      59.599  -1.097  38.964  1.00 24.82           C
ATOM   1890  O   HIS A 223      59.723  -2.283  39.270  1.00 24.66           O
ATOM   1891  CB  HIS A 223      58.465   0.637  40.359  1.00 19.53           C
ATOM   1892  CG  HIS A 223      57.373  -0.333  40.759  1.00 28.64           C
ATOM   1893  ND1 HIS A 223      57.021  -0.564  42.082  1.00 24.16           N
ATOM   1894  CD2 HIS A 223      56.497  -1.062  40.004  1.00 30.39           C
ATOM   1895  CE1 HIS A 223      55.983  -1.399  42.112  1.00 30.39           C
ATOM   1896  NE2 HIS A 223      55.652  -1.727  40.869  1.00 28.13           N
ATOM   1897  N   VAL A 224      59.354  -0.684  37.725  1.00 22.06           N
ATOM   1898  CA  VAL A 224      59.111  -1.657  36.652  1.00 19.15           C
ATOM   1899  C   VAL A 224      60.350  -2.490  36.333  1.00 25.89           C
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ATOM   1900  O   VAL A 224      60.282  -3.709  36.250  1.00 22.37           O
ATOM   1901  CB  VAL A 224      58.559  -1.022  35.377  1.00 22.59           C
ATOM   1902  CG1 VAL A 224      58.512  -2.050  34.231  1.00 22.61           C
ATOM   1903  CG2 VAL A 224      57.161  -0.491  35.650  1.00 23.44           C
ATOM   1904  N   MET A 225      61.499  -1.838  36.255  1.00 27.83           N
ATOM   1905  CA  MET A 225      62.710  -2.577  36.004  1.00 23.69           C
ATOM   1906  C   MET A 225      62.896  -3.678  37.071  1.00 31.95           C
ATOM   1907  O   MET A 225      63.290  -4.805  36.785  1.00 24.33           O
ATOM   1908  CB  MET A 225      63.902  -1.604  36.056  1.00 21.34           C
ATOM   1909  CG  MET A 225      65.295  -2.296  35.999  1.00 17.83           C
ATOM   1910  SD  MET A 225      65.750  -2.958  34.306  1.00 23.33           S
ATOM   1911  CE  MET A 225      67.080  -1.896  33.785  1.00 16.46           C
ATOM   1912  N   GLU A 226      62.644  -3.319  38.316  1.00 19.54           N
ATOM   1913  CA  GLU A 226      62.988  -4.161  39.428  1.00 21.58           C
ATOM   1914  C   GLU A 226      61.999  -5.200  39.918  1.00 30.77           C
ATOM   1915  O   GLU A 226      62.308  -6.012  40.780  1.00 29.39           O
ATOM   1916  CB  GLU A 226      63.613  -3.323  40.547  1.00 20.47           C
ATOM   1917  CG  GLU A 226      64.937  -2.673  40.122  1.00 23.03           C
ATOM   1918  CD  GLU A 226      65.504  -1.809  41.208  1.00 32.62           C
ATOM   1919  OE1 GLU A 226      64.721  -1.455  42.122  1.00 26.12           O
ATOM   1920  OE2 GLU A 226      66.711  -1.479  41.152  1.00 17.67           O
ATOM   1921  N   LEU A 227      60.837  -5.248  39.295  1.00 34.11           N
ATOM   1922  CA  LEU A 227      59.883  -6.296  39.642  1.00 35.26           C
ATOM   1923  C   LEU A 227      60.537  -7.644  39.320  1.00 27.91           C
ATOM   1924  O   LEU A 227      61.291  -7.766  38.340  1.00 19.89           O
ATOM   1925  CB  LEU A 227      58.693  -6.236  38.678  1.00 36.48           C
ATOM   1926  CG  LEU A 227      57.381  -5.569  39.155  1.00 40.30           C
ATOM   1927  CD1 LEU A 227      57.697  -4.194  39.382  1.00 42.04           C
ATOM   1928  CD2 LEU A 227      56.610  -5.577  37.647  1.00 46.21           C
ATOM   1929  N   ALA A 228      60.026  -8.688  39.955  1.00 27.15           N
ATOM   1930  CA  ALA A 228      60.425 -10.051  39.616  1.00 25.26           C
ATOM   1931  C   ALA A 228      59.801 -10.435  38.279  1.00 27.93           C
ATOM   1932  O   ALA A 228      58.624 -10.093  37.934  1.00 31.26           O
ATOM   1933  CB  ALA A 228      60.003 -11.052  40.703  1.00 22.05           C
ATOM   1934  N   HIS A 229      60.624 -11.160  37.539  1.00 27.05           N
ATOM   1935  CA  HIS A 229      60.275 -11.605  36.222  1.00 24.42           C
ATOM   1936  C   HIS A 229      58.905 -12.260  36.184  1.00 21.74           C
ATOM   1937  O   HIS A 229      58.015 -11.851  35.398  1.00 22.22           O
ATOM   1938  CB  HIS A 229      61.351 -12.520  35.698  1.00 17.71           C
ATOM   1939  CG  HIS A 229      61.284 -12.701  34.220  1.00 27.24           C
ATOM   1940  ND1 HIS A 229      61.060 -11.650  33.350  1.00 34.38           N
ATOM   1941  CD2 HIS A 229      61.292 -13.821  33.465  1.00 31.45           C
ATOM   1942  CE1 HIS A 229      60.992 -12.113  32.115  1.00 30.50           C
ATOM   1943  NE2 HIS A 229      61.124 -13.427  32.159  1.00 35.23           N
ATOM   1944  N   GLU A 230      58.681 -13.161  37.140  1.00 20.24           N
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ATOM 1945 CA GLU A 230 57.425 -13.895 37.209 1.00 29.41 C
ATOM 1946 C GLU A 230 56.181 -13.051 37.341 1.00 22.20 C
ATOM 1947 O GLU A 230 55.159 -13.359 36.679 1.00 17.78 O
ATOM 1948 CB GLU A 230 57.464 -14.997 38.274 1.00 38.51 C
ATOM 1949 CG GLU A 230 58.085 -14.582 39.567 1.00 63.09 C
ATOM 1950 CD GLU A 230 57.036 -14.473 40.661 1.00100.00 C
ATOM 1951 OE1 GLU A 230 55.859 -14.872 40.400 1.00100.00 O
ATOM 1952 OE2 GLU A 230 57.409 -14.003 41.768 1.00 81.48 O
ATOM 1953 N VAL A 231 56.272 -12.004 38.182 1.00 16.53 N
ATOM 1954 CA VAL A 231 55.202 -11.029 38.356 1.00 20.23 C
ATOM 1955 C VAL A 231 55.009 -10.164 37.102 1.00 24.45 C
ATOM 1956 O VAL A 231 53.864 -9.834 36.705 1.00 21.00 O
ATOM 1957 CB VAL A 231 55.541 -10.057 39.426 1.00 28.61 C
ATOM 1958 CG1 VAL A 231 54.362 -9.098 39.610 1.00 29.78 C
ATOM 1959 CG2 VAL A 231 55.881 -10.757 40.677 1.00 28.96 C
ATOM 1960 N TRP A 232 56.133 -9.798 36.486 1.00 17.17 N
ATOM 1961 CA TRP A 232 56.052 -9.044 35.262 1.00 21.52 C
ATOM 1962 C TRP A 232 55.388 -9.844 34.156 1.00 20.53 C
ATOM 1963 O TRP A 232 54.588 -9.306 33.380 1.00 24.31 O
ATOM 1964 CB TRP A 232 57.438 -8.644 34.801 1.00 29.88 C
ATOM 1965 CG TRP A 232 57.430 -7.843 33.500 1.00 27.65 C
ATOM 1966 CD1 TRP A 232 57.184 -6.464 33.356 1.00 25.42 C
ATOM 1967 CD2 TRP A 232 57.714 -8.336 32.169 1.00 27.75 C
ATOM 1968 NE1 TRP A 232 57.325 -6.095 32.033 1.00 22.53 N
ATOM 1969 CE2 TRP A 232 57.655 -7.203 31.279 1.00 25.11 C
ATOM 1970 CE3 TRP A 232 58.037 -9.603 31.640 1.00 22.72 C
ATOM 1971 CZ2 TRP A 232 57.917 -7.316 29.879 1.00 17.23 C
ATOM 1972 CZ3 TRP A 232 58.238 -9.720 30.223 1.00 25.97 C
ATOM 1973 CH2 TRP A 232 58.154 -8.581 29.368 1.00 22.07 C
ATOM 1974 N LEU A 233 55.749 -11.121 34.018 1.00 23.80 N
ATOM 1975 CA LEU A 233 55.141 -11.949 32.937 1.00 24.78 C
ATOM 1976 C LEU A 233 53.652 -12.118 33.122 1.00 24.51 C
ATOM 1977 O LEU A 233 52.865 -12.075 32.163 1.00 28.50 O
ATOM 1978 CB LEU A 233 55.765 -13.348 32.820 1.00 26.20 C
ATOM 1979 CG LEU A 233 57.250 -13.505 32.503 1.00 19.39 C
ATOM 1980 CD1 LEU A 233 57.745 -14.850 33.023 1.00 19.90 C
ATOM 1981 CD2 LEU A 233 57.561 -13.287 31.017 1.00 16.01 C
ATOM 1982 N GLU A 234 53.298 -12.343 34.372 1.00 25.45 N
ATOM 1983 CA GLU A 234 51.929 -12.523 34.822 1.00 30.04 C
ATOM 1984 C GLU A 234 51.128 -11.319 34.367 1.00 35.69 C
ATOM 1985 O GLU A 234 49.926 -11.390 34.052 1.00 28.25 O
ATOM 1986 CB GLU A 234 52.007 -12.468 36.344 1.00 37.30 C
ATOM 1987 CG GLU A 234 50.908 -13.133 37.118 1.00 45.39 C
ATOM 1988 CD GLU A 234 51.112 -12.881 38.601 1.00100.00 C
ATOM 1989 OE1 GLU A 234 52.240 -13.137 39.104 1.00 99.09 O

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ATOM   1990  OE2 GLU A 234      50.211 -12.257  39.211  1.00100.00           O
ATOM   1991  N   ASN A 235      51.802 -10.184  34.364  1.00 25.04           N
ATOM   1992  CA  ASN A 235      51.109  -8.986  33.992  1.00 26.17           C
ATOM   1993  C   ASN A 235      51.280  -8.494  32.571  1.00 30.46           C
ATOM   1994  O   ASN A 235      50.824  -7.393  32.259  1.00 22.90           O
ATOM   1995  CB  ASN A 235      51.427  -7.895  34.981  1.00 29.23           C
ATOM   1996  CG  ASN A 235      50.878  -8.197  36.342  1.00 39.27           C
ATOM   1997  OD1 ASN A 235      49.722  -7.882  36.628  1.00 29.06           O
ATOM   1998  ND2 ASN A 235      51.653  -8.934  37.140  1.00 40.22           N
ATOM   1999  N   THR A 236      51.935  -9.268  31.708  1.00 20.97           N
ATOM   2000  CA  THR A 236      52.108  -8.795  30.344  1.00 22.30           C
ATOM   2001  C   THR A 236      51.867  -9.943  29.419  1.00 29.74           C
ATOM   2002  O   THR A 236      51.551 -11.033  29.895  1.00 21.23           O
ATOM   2003  CB  THR A 236      53.545  -8.306  30.161  1.00 22.73           C
ATOM   2004  OG1 THR A 236      54.422  -9.325  30.636  1.00 21.23           O
ATOM   2005  CG2 THR A 236      53.801  -7.048  31.041  1.00 19.69           C
ATOM   2006  N   GLN A 237      52.003  -9.699  28.109  1.00 22.23           N
ATOM   2007  CA  GLN A 237      52.097 -10.783  27.122  1.00 16.69           C
ATOM   2008  C   GLN A 237      53.335 -10.507  26.331  1.00 21.02           C
ATOM   2009  O   GLN A 237      53.729  -9.362  26.204  1.00 22.19           O
ATOM   2010  CB  GLN A 237      50.913 -10.999  26.189  1.00  8.23           C
ATOM   2011  CG  GLN A 237      49.639 -11.096  26.904  1.00 21.04           C
ATOM   2012  CD  GLN A 237      48.907  -9.862  26.606  1.00 62.07           C
ATOM   2013  OE1 GLN A 237      48.437  -9.712  25.460  1.00 59.32           O
ATOM   2014  NE2 GLN A 237      49.220  -8.847  27.388  1.00 37.82           N
ATOM   2015  N   PRO A 238      54.002 -11.579  25.917  1.00 28.76           N
ATOM   2016  CA  PRO A 238      55.275 -11.438  25.246  1.00 30.28           C
ATOM   2017  C   PRO A 238      55.194 -10.643  23.958  1.00 29.08           C
ATOM   2018  O   PRO A 238      56.181 -10.029  23.600  1.00 15.95           O
ATOM   2019  CB  PRO A 238      55.733 -12.879  25.011  1.00 22.54           C
ATOM   2020  CG  PRO A 238      54.898 -13.710  25.886  1.00 18.92           C
ATOM   2021  CD  PRO A 238      53.626 -12.998  26.068  1.00 11.75           C
ATOM   2022  N   MET A 239      54.041 -10.635  23.286  1.00 17.26           N
ATOM   2023  CA  MET A 239      53.924  -9.807  22.104  1.00 17.85           C
ATOM   2024  C   MET A 239      53.109  -8.509  22.362  1.00 18.63           C
ATOM   2025  O   MET A 239      52.792  -7.741  21.419  1.00 16.82           O
ATOM   2026  CB  MET A 239      53.460 -10.588  20.881  1.00 15.22           C
ATOM   2027  CG  MET A 239      54.536 -11.534  20.261  1.00 12.90           C
ATOM   2028  SD  MET A 239      53.994 -12.534  18.808  1.00 17.49           S
ATOM   2029  CE  MET A 239      54.350 -11.357  17.422  1.00 13.12           C
ATOM   2030  N   LEU A 240      52.847  -8.252  23.646  1.00 18.55           N
ATOM   2031  CA  LEU A 240      52.159  -7.037  24.131  1.00 16.68           C
ATOM   2032  C   LEU A 240      52.774  -6.733  25.493  1.00 11.82           C
ATOM   2033  O   LEU A 240      52.124  -6.803  26.549  1.00 13.84           O
ATOM   2034  CB  LEU A 240      50.645  -7.249  24.240  1.00 16.91           C
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ATOM	2035	CG	LEU	A	240	49.646	-6.120	23.852	1.00	22.29	C
ATOM	2036	CD1	LEU	A	240	48.968	-5.488	25.033	1.00	25.51	C
ATOM	2037	CD2	LEU	A	240	50.070	-5.059	22.815	1.00	28.07	C
ATOM	2038	N	SER	A	241	54.076	-6.467	25.456	1.00	13.09	N
ATOM	2039	CA	SER	A	241	54.842	-6.315	26.682	1.00	24.20	C
ATOM	2040	C	SER	A	241	54.947	-4.938	27.377	1.00	30.52	C
ATOM	2041	O	SER	A	241	55.363	-4.854	28.547	1.00	17.02	O
ATOM	2042	CB	SER	A	241	56.247	-6.900	26.495	1.00	14.04	C
ATOM	2043	OG	SER	A	241	57.062	-6.144	25.598	1.00	13.95	O
ATOM	2044	N	HIS	A	242	54.661	-3.861	26.659	1.00	17.87	N
ATOM	2045	CA	HIS	A	242	54.894	-2.548	27.221	1.00	13.55	C
ATOM	2046	C	HIS	A	242	53.990	-2.254	28.373	1.00	13.70	C
ATOM	2047	O	HIS	A	242	52.974	-2.885	28.539	1.00	13.29	O
ATOM	2048	CB	HIS	A	242	54.826	-1.430	26.130	1.00	16.05	C
ATOM	2049	CG	HIS	A	242	53.595	-1.504	25.272	1.00	18.88	C
ATOM	2050	ND1	HIS	A	242	52.591	-0.553	25.326	1.00	23.24	N
ATOM	2051	CD2	HIS	A	242	53.165	-2.461	24.413	1.00	13.19	C
ATOM	2052	CE1	HIS	A	242	51.629	-0.887	24.483	1.00	17.44	C
ATOM	2053	NE2	HIS	A	242	51.962	-2.031	23.901	1.00	19.54	N
ATOM	2054	N	ILE	A	243	54.310	-1.203	29.095	1.00	15.84	N
ATOM	2055	CA	ILE	A	243	53.492	-0.809	30.192	1.00	19.10	C
ATOM	2056	C	ILE	A	243	53.336	0.714	30.191	1.00	23.23	C
ATOM	2057	O	ILE	A	243	54.312	1.406	30.385	1.00	12.10	O
ATOM	2058	CB	ILE	A	243	54.166	-1.273	31.482	1.00	24.62	C
ATOM	2059	CG1	ILE	A	243	54.014	-2.783	31.576	1.00	25.60	C
ATOM	2060	CG2	ILE	A	243	53.497	-0.665	32.735	1.00	17.37	C
ATOM	2061	CD1	ILE	A	243	54.725	-3.365	32.714	1.00	14.82	C
ATOM	2062	N	ASN	A	244	52.112	1.217	30.013	1.00	16.43	N
ATOM	2063	CA	ASN	A	244	51.824	2.689	30.038	1.00	18.99	C
ATOM	2064	C	ASN	A	244	52.252	3.292	31.348	1.00	18.83	C
ATOM	2065	O	ASN	A	244	51.965	2.727	32.405	1.00	19.58	O
ATOM	2066	CB	ASN	A	244	50.304	2.987	29.910	1.00	15.67	C
ATOM	2067	CG	ASN	A	244	49.768	2.702	28.517	1.00	14.57	C
ATOM	2068	OD1	ASN	A	244	50.546	2.583	27.580	1.00	13.64	O
ATOM	2069	ND2	ASN	A	244	48.443	2.491	28.393	1.00	10.16	N
ATOM	2070	N	VAL	A	245	52.800	4.499	31.326	1.00	13.50	N
ATOM	2071	CA	VAL	A	245	53.159	5.134	32.602	1.00	13.49	C
ATOM	2072	C	VAL	A	245	52.528	6.566	32.644	1.00	16.25	C
ATOM	2073	O	VAL	A	245	52.786	7.405	31.770	1.00	15.20	O
ATOM	2074	CB	VAL	A	245	54.754	5.163	32.810	1.00	21.07	C
ATOM	2075	CG1	VAL	A	245	55.154	6.085	33.937	1.00	15.08	C
ATOM	2076	CG2	VAL	A	245	55.280	3.817	33.143	1.00	15.82	C
ATOM	2077	N	GLY	A	246	51.696	6.843	33.649	1.00	14.03	N
ATOM	2078	CA	GLY	A	246	51.027	8.136	33.707	1.00	16.87	C
ATOM	2079	C	GLY	A	246	50.146	8.203	34.939	1.00	26.95	C

ATOM	2080	O	GLY	A	246	50.323	7.401	35.850	1.00	23.04	O
ATOM	2081	N	THR	A	247	49.207	9.161	34.963	1.00	21.44	N
ATOM	2082	CA	THR	A	247	48.232	9.276	36.063	1.00	21.39	C
ATOM	2083	C	THR	A	247	46.868	8.677	35.673	1.00	24.08	C
ATOM	2084	O	THR	A	247	46.069	8.306	36.508	1.00	21.03	O
ATOM	2085	CB	THR	A	247	47.988	10.730	36.404	1.00	22.24	C
ATOM	2086	OG1	THR	A	247	47.409	11.389	35.265	1.00	18.62	O
ATOM	2087	CG2	THR	A	247	49.275	11.378	36.724	1.00	18.99	C
ATOM	2088	N	GLY	A	248	46.583	8.651	34.384	1.00	24.95	N
ATOM	2089	CA	GLY	A	248	45.319	8.143	33.924	1.00	22.61	C
ATOM	2090	C	GLY	A	248	44.223	9.160	34.226	1.00	21.42	C
ATOM	2091	O	GLY	A	248	43.059	8.866	34.137	1.00	25.70	O
ATOM	2092	N	VAL	A	249	44.615	10.386	34.521	1.00	30.72	N
ATOM	2093	CA	VAL	A	249	43.673	11.464	34.827	1.00	26.09	C
ATOM	2094	C	VAL	A	249	43.747	12.596	33.786	1.00	32.70	C
ATOM	2095	O	VAL	A	249	44.853	13.006	33.387	1.00	26.92	O
ATOM	2096	CB	VAL	A	249	44.020	12.085	36.214	1.00	38.59	C
ATOM	2097	CG1	VAL	A	249	43.225	13.324	36.470	1.00	36.11	C
ATOM	2098	CG2	VAL	A	249	43.782	11.083	37.306	1.00	41.30	C
ATOM	2099	N	ASP	A	250	42.581	13.125	33.397	1.00	27.95	N
ATOM	2100	CA	ASP	A	250	42.488	14.232	32.439	1.00	20.64	C
ATOM	2101	C	ASP	A	250	42.611	15.581	33.155	1.00	27.63	C
ATOM	2102	O	ASP	A	250	42.188	15.783	34.308	1.00	26.23	O
ATOM	2103	CB	ASP	A	250	41.075	14.302	31.827	1.00	23.89	C
ATOM	2104	CG	ASP	A	250	40.768	13.180	30.850	1.00	39.52	C
ATOM	2105	OD1	ASP	A	250	41.283	13.184	29.688	1.00	39.96	O
ATOM	2106	OD2	ASP	A	250	39.767	12.501	31.153	1.00	45.34	O
ATOM	2107	N	CYS	A	251	43.029	16.566	32.388	1.00	20.12	N
ATOM	2108	CA	CYS	A	251	42.962	17.906	32.851	1.00	27.20	C
ATOM	2109	C	CYS	A	251	42.918	18.779	31.577	1.00	26.47	C
ATOM	2110	O	CYS	A	251	43.699	18.560	30.633	1.00	19.45	O
ATOM	2111	CB	CYS	A	251	44.148	18.157	33.778	1.00	34.86	C
ATOM	2112	SG	CYS	A	251	45.129	19.619	33.453	1.00	29.47	S
ATOM	2113	N	THR	A	252	41.932	19.673	31.494	1.00	14.85	N
ATOM	2114	CA	THR	A	252	41.834	20.588	30.335	1.00	21.21	C
ATOM	2115	C	THR	A	252	42.999	21.592	30.236	1.00	20.53	C
ATOM	2116	O	THR	A	252	43.657	21.926	31.249	1.00	15.24	O
ATOM	2117	CB	THR	A	252	40.506	21.407	30.329	1.00	32.08	C
ATOM	2118	OG1	THR	A	252	40.460	22.304	31.447	1.00	19.26	O
ATOM	2119	CG2	THR	A	252	39.309	20.495	30.372	1.00	13.91	C
ATOM	2120	N	ILE	A	253	43.228	22.095	29.024	1.00	14.81	N
ATOM	2121	CA	ILE	A	253	44.264	23.118	28.812	1.00	16.90	C
ATOM	2122	C	ILE	A	253	43.934	24.383	29.627	1.00	23.41	C
ATOM	2123	O	ILE	A	253	44.834	25.012	30.247	1.00	15.27	O
ATOM	2124	CB	ILE	A	253	44.404	23.452	27.302	1.00	24.05	C

ATOM 2125 CG1 ILE A 253 44.862 22.200 26.561 1.00 27.33 C
ATOM 2126 CG2 ILE A 253 45.473 24.479 27.077 1.00 9.22 C
ATOM 2127 CD1 ILE A 253 45.662 21.276 27.452 1.00 49.56 C
ATOM 2128 N ARG A 254 42.637 24.709 29.707 1.00 19.56 N
ATOM 2129 CA ARG A 254 42.228 25.865 30.522 1.00 19.41 C
ATOM 2130 C ARG A 254 42.712 25.713 31.970 1.00 18.10 C
ATOM 2131 O ARG A 254 43.311 26.616 32.515 1.00 13.89 O
ATOM 2132 CB ARG A 254 40.704 26.101 30.480 1.00 15.98 C
ATOM 2133 CG ARG A 254 40.282 27.378 31.255 1.00 9.96 C
ATOM 2134 CD ARG A 254 38.809 27.702 31.218 1.00 24.79 C
ATOM 2135 NE ARG A 254 38.498 28.414 29.997 1.00 29.42 N
ATOM 2136 CZ ARG A 254 38.693 29.723 29.794 1.00 59.85 C
ATOM 2137 NH1 ARG A 254 39.194 30.527 30.732 1.00 42.58 N
ATOM 2138 NH2 ARG A 254 38.377 30.245 28.620 1.00 18.44 N
ATOM 2139 N ASP A 255 42.406 24.564 32.586 1.00 20.22 N
ATOM 2140 CA ASP A 255 42.795 24.205 33.974 1.00 16.48 C
ATOM 2141 C ASP A 255 44.321 24.372 34.069 1.00 22.43 C
ATOM 2142 O ASP A 255 44.868 24.897 35.060 1.00 18.53 O
ATOM 2143 CB ASP A 255 42.478 22.686 34.157 1.00 19.17 C
ATOM 2144 CG ASP A 255 42.144 22.246 35.610 1.00 47.08 C
ATOM 2145 OD1 ASP A 255 41.780 23.090 36.429 1.00 49.66 O
ATOM 2146 OD2 ASP A 255 42.020 21.016 35.880 1.00 48.12 O
ATOM 2147 N LEU A 256 45.014 23.809 33.078 1.00 15.98 N
ATOM 2148 CA LEU A 256 46.465 23.844 33.069 1.00 21.76 C
ATOM 2149 C LEU A 256 47.020 25.275 33.076 1.00 16.79 C
ATOM 2150 O LEU A 256 47.825 25.697 33.946 1.00 15.24 O
ATOM 2151 CB LEU A 256 46.967 23.056 31.859 1.00 23.33 C
ATOM 2152 CG LEU A 256 48.491 23.100 31.765 1.00 26.80 C
ATOM 2153 CD1 LEU A 256 49.171 22.334 32.984 1.00 17.13 C
ATOM 2154 CD2 LEU A 256 49.040 22.724 30.346 1.00 15.42 C
ATOM 2155 N ALA A 257 46.520 26.048 32.140 1.00 13.77 N
ATOM 2156 CA ALA A 257 46.938 27.436 32.025 1.00 12.70 C
ATOM 2157 C ALA A 257 46.656 28.237 33.267 1.00 10.73 C
ATOM 2158 O ALA A 257 47.451 29.073 33.672 1.00 20.33 O
ATOM 2159 CB ALA A 257 46.208 28.073 30.834 1.00 13.34 C
ATOM 2160 N GLN A 258 45.470 28.080 33.835 1.00 12.40 N
ATOM 2161 CA GLN A 258 45.102 28.911 34.981 1.00 8.39 C
ATOM 2162 C GLN A 258 45.879 28.480 36.166 1.00 13.48 C
ATOM 2163 O GLN A 258 46.178 29.281 37.029 1.00 22.96 O
ATOM 2164 CB GLN A 258 43.614 28.761 35.305 1.00 16.12 C
ATOM 2165 CG GLN A 258 42.674 29.096 34.130 1.00 30.19 C
ATOM 2166 CD GLN A 258 42.574 30.585 33.781 1.00 37.29 C
ATOM 2167 OE1 GLN A 258 42.911 31.471 34.610 1.00 21.24 O
ATOM 2168 NE2 GLN A 258 42.021 30.876 32.572 1.00 15.94 N
ATOM 2169 N THR A 259 46.179 27.182 36.232 1.00 16.21 N

ATOM 2170 CA THR A 259 46.982 26.678 37.336 1.00 16.85 C
ATOM 2171 C THR A 259 48.410 27.186 37.233 1.00 20.56 C
ATOM 2172 O THR A 259 49.002 27.621 38.214 1.00 21.44 O
ATOM 2173 CB THR A 259 47.066 25.192 37.361 1.00 27.56 C
ATOM 2174 OG1 THR A 259 45.752 24.620 37.509 1.00 20.92 O
ATOM 2175 CG2 THR A 259 47.936 24.796 38.545 1.00 12.85 C
ATOM 2176 N ILE A 260 48.952 27.170 36.028 1.00 19.96 N
ATOM 2177 CA ILE A 260 50.292 27.704 35.839 1.00 23.01 C
ATOM 2178 C ILE A 260 50.313 29.180 36.225 1.00 31.73 C
ATOM 2179 O ILE A 260 51.211 29.627 36.993 1.00 25.90 O
ATOM 2180 CB ILE A 260 50.835 27.456 34.390 1.00 22.46 C
ATOM 2181 CG1 ILE A 260 51.153 25.940 34.232 1.00 24.12 C
ATOM 2182 CG2 ILE A 260 52.099 28.361 34.106 1.00 13.47 C
ATOM 2183 CD1 ILE A 260 51.501 25.443 32.810 1.00 12.58 C
ATOM 2184 N ALA A 261 49.280 29.910 35.764 1.00 15.35 N
ATOM 2185 CA ALA A 261 49.177 31.355 36.048 1.00 16.00 C
ATOM 2186 C ALA A 261 49.316 31.604 37.550 1.00 20.58 C
ATOM 2187 O ALA A 261 50.104 32.443 37.987 1.00 16.09 O
ATOM 2188 CB ALA A 261 47.832 31.958 35.487 1.00 13.65 C
ATOM 2189 N LYS A 262 48.551 30.843 38.323 1.00 11.50 N
ATOM 2190 CA LYS A 262 48.578 30.905 39.770 1.00 10.13 C
ATOM 2191 C LYS A 262 49.968 30.460 40.296 1.00 28.08 C
ATOM 2192 O LYS A 262 50.503 31.084 41.205 1.00 29.37 O
ATOM 2193 CB LYS A 262 47.453 30.032 40.335 1.00 12.50 C
ATOM 2194 CG LYS A 262 47.332 29.962 41.888 1.00 16.51 C
ATOM 2195 CD LYS A 262 46.092 29.092 42.371 1.00 46.61 C
ATOM 2196 CE LYS A 262 46.344 27.555 42.661 1.00 99.70 C
ATOM 2197 NZ LYS A 262 45.157 26.703 43.200 1.00 36.59 N
ATOM 2198 N VAL A 263 50.589 29.443 39.705 1.00 17.44 N
ATOM 2199 CA VAL A 263 51.915 29.039 40.171 1.00 18.72 C
ATOM 2200 C VAL A 263 52.997 30.170 39.997 1.00 32.12 C
ATOM 2201 O VAL A 263 53.871 30.412 40.834 1.00 21.18 O
ATOM 2202 CB VAL A 263 52.389 27.709 39.476 1.00 16.35 C
ATOM 2203 CG1 VAL A 263 53.920 27.518 39.647 1.00 11.83 C
ATOM 2204 CG2 VAL A 263 51.646 26.522 40.093 1.00 14.99 C
ATOM 2205 N VAL A 264 52.913 30.899 38.909 1.00 21.75 N
ATOM 2206 CA VAL A 264 53.917 31.877 38.653 1.00 19.81 C
ATOM 2207 C VAL A 264 53.719 33.208 39.377 1.00 35.79 C
ATOM 2208 O VAL A 264 54.632 34.032 39.482 1.00 28.99 O
ATOM 2209 CB VAL A 264 54.059 32.014 37.175 1.00 24.27 C
ATOM 2210 CG1 VAL A 264 54.728 33.269 36.822 1.00 33.58 C
ATOM 2211 CG2 VAL A 264 54.840 30.808 36.674 1.00 23.01 C
ATOM 2212 N GLY A 265 52.550 33.378 39.969 1.00 25.30 N
ATOM 2213 CA GLY A 265 52.241 34.620 40.636 1.00 24.14 C
ATOM 2214 C GLY A 265 51.730 35.694 39.632 1.00 35.03 C

ATOM	2215	O	GLY A 265	51.773	36.911	39.962	1.00 33.71	O
ATOM	2216	N	TYR A 266	51.294	35.257	38.428	1.00 26.25	N
ATOM	2217	CA	TYR A 266	50.698	36.151	37.373	1.00 26.55	C
ATOM	2218	C	TYR A 266	49.364	36.745	37.818	1.00 31.01	C
ATOM	2219	O	TYR A 266	48.532	36.067	38.456	1.00 27.99	O
ATOM	2220	CB	TYR A 266	50.501	35.463	36.008	1.00 24.31	C
ATOM	2221	CG	TYR A 266	49.994	36.381	34.884	1.00 28.64	C
ATOM	2222	CD1	TYR A 266	50.670	37.582	34.542	1.00 35.05	C
ATOM	2223	CD2	TYR A 266	48.860	36.038	34.118	1.00 22.60	C
ATOM	2224	CE1	TYR A 266	50.212	38.434	33.472	1.00 20.73	C
ATOM	2225	CE2	TYR A 266	48.428	36.859	33.012	1.00 20.91	C
ATOM	2226	CZ	TYR A 266	49.088	38.062	32.735	1.00 23.85	C
ATOM	2227	OH	TYR A 266	48.622	38.851	31.710	1.00 33.40	O
ATOM	2228	N	LYS A 267	49.217	38.043	37.604	1.00 25.72	N
ATOM	2229	CA	LYS A 267	47.988	38.697	38.009	1.00 30.77	C
ATOM	2230	C	LYS A 267	47.217	39.280	36.798	1.00 28.85	C
ATOM	2231	O	LYS A 267	46.179	39.894	36.949	1.00 31.17	O
ATOM	2232	CB	LYS A 267	48.279	39.741	39.092	1.00 27.13	C
ATOM	2233	CG	LYS A 267	48.728	39.128	40.403	1.00 23.18	C
ATOM	2234	CD	LYS A 267	48.420	40.096	41.562	1.00 30.98	C
ATOM	2235	CE	LYS A 267	47.933	39.358	42.820	1.00 48.52	C
ATOM	2236	NZ	LYS A 267	47.005	38.208	42.505	1.00100.00	N
ATOM	2237	N	GLY A 268	47.716	39.054	35.594	1.00 22.67	N
ATOM	2238	CA	GLY A 268	47.019	39.518	34.394	1.00 21.38	C
ATOM	2239	C	GLY A 268	45.856	38.568	34.085	1.00 31.03	C
ATOM	2240	O	GLY A 268	45.455	37.728	34.911	1.00 19.71	O
ATOM	2241	N	ARG A 269	45.387	38.645	32.849	1.00 30.40	N
ATOM	2242	CA	ARG A 269	44.263	37.846	32.399	1.00 26.47	C
ATOM	2243	C	ARG A 269	44.680	36.705	31.489	1.00 22.35	C
ATOM	2244	O	ARG A 269	45.378	36.926	30.524	1.00 22.75	O
ATOM	2245	CB	ARG A 269	43.297	38.753	31.626	1.00 22.65	C
ATOM	2246	CG	ARG A 269	42.201	39.390	32.463	1.00 24.21	C
ATOM	2247	CD	ARG A 269	40.936	39.465	31.568	1.00 83.45	C
ATOM	2248	NE	ARG A 269	40.113	40.676	31.762	1.00100.00	N
ATOM	2249	CZ	ARG A 269	38.808	40.751	31.431	1.00100.00	C
ATOM	2250	NH1	ARG A 269	38.201	39.691	30.921	1.00 99.93	N
ATOM	2251	NH2	ARG A 269	38.094	41.865	31.663	1.00100.00	N
ATOM	2252	N	VAL A 270	44.195	35.494	31.758	1.00 19.87	N
ATOM	2253	CA	VAL A 270	44.468	34.389	30.856	1.00 24.82	C
ATOM	2254	C	VAL A 270	43.319	34.456	29.824	1.00 22.51	C
ATOM	2255	O	VAL A 270	42.145	34.501	30.181	1.00 25.79	O
ATOM	2256	CB	VAL A 270	44.436	32.979	31.571	1.00 24.03	C
ATOM	2257	CG1	VAL A 270	44.576	31.861	30.533	1.00 20.72	C
ATOM	2258	CG2	VAL A 270	45.506	32.849	32.639	1.00 11.27	C
ATOM	2259	N	VAL A 271	43.660	34.409	28.554	1.00 25.18	N

ATOM 2260 CA VAL A 271 42.666 34.492 27.487 1.00 28.32 C
ATOM 2261 C VAL A 271 42.819 33.370 26.442 1.00 24.89 C
ATOM 2262 O VAL A 271 43.923 33.115 25.980 1.00 21.98 O
ATOM 2263 CB VAL A 271 42.901 35.813 26.736 1.00 29.25 C
ATOM 2264 CG1 VAL A 271 42.256 35.773 25.370 1.00 31.91 C
ATOM 2265 CG2 VAL A 271 42.421 36.989 27.565 1.00 18.72 C
ATOM 2266 N PHE A 272 41.716 32.758 26.019 1.00 26.14 N
ATOM 2267 CA PHE A 272 41.752 31.747 24.963 1.00 24.34 C
ATOM 2268 C PHE A 272 41.236 32.266 23.623 1.00 28.95 C
ATOM 2269 O PHE A 272 40.155 32.826 23.582 1.00 22.01 O
ATOM 2270 CB PHE A 272 40.960 30.506 25.391 1.00 20.97 C
ATOM 2271 CG PHE A 272 41.764 29.570 26.243 1.00 21.77 C
ATOM 2272 CD1 PHE A 272 41.940 29.842 27.610 1.00 14.60 C
ATOM 2273 CD2 PHE A 272 42.504 28.550 25.656 1.00 22.19 C
ATOM 2274 CE1 PHE A 272 42.763 29.041 28.434 1.00 17.89 C
ATOM 2275 CE2 PHE A 272 43.336 27.726 26.454 1.00 27.64 C
ATOM 2276 CZ PHE A 272 43.478 27.979 27.851 1.00 25.14 C
ATOM 2277 N ASP A 273 42.012 32.114 22.542 1.00 29.45 N
ATOM 2278 CA ASP A 273 41.557 32.536 21.214 1.00 22.33 C
ATOM 2279 C ASP A 273 40.896 31.365 20.493 1.00 25.67 C
ATOM 2280 O ASP A 273 41.539 30.570 19.793 1.00 17.81 O
ATOM 2281 CB ASP A 273 42.672 33.114 20.343 1.00 21.45 C
ATOM 2282 CG ASP A 273 42.131 33.626 18.990 1.00 26.89 C
ATOM 2283 OD1 ASP A 273 40.975 33.249 18.598 1.00 27.76 O
ATOM 2284 OD2 ASP A 273 42.838 34.421 18.327 1.00 30.06 O
ATOM 2285 N ALA A 274 39.589 31.284 20.649 1.00 15.59 N
ATOM 2286 CA ALA A 274 38.932 30.128 20.128 1.00 23.75 C
ATOM 2287 C ALA A 274 38.853 30.168 18.653 1.00 32.30 C
ATOM 2288 O ALA A 274 38.284 29.256 18.029 1.00 29.37 O
ATOM 2289 CB ALA A 274 37.567 29.905 20.777 1.00 18.87 C
ATOM 2290 N SER A 275 39.372 31.243 18.081 1.00 21.10 N
ATOM 2291 CA SER A 275 39.343 31.288 16.631 1.00 26.90 C
ATOM 2292 C SER A 275 40.390 30.300 16.116 1.00 43.37 C
ATOM 2293 O SER A 275 40.421 29.949 14.927 1.00 46.32 O
ATOM 2294 CB SER A 275 39.547 32.683 16.074 1.00 15.19 C
ATOM 2295 OG SER A 275 40.904 33.070 16.078 1.00 28.71 O
ATOM 2296 N LYS A 276 41.192 29.780 17.037 1.00 22.98 N
ATOM 2297 CA LYS A 276 42.178 28.791 16.638 1.00 23.28 C
ATOM 2298 C LYS A 276 41.645 27.405 16.976 1.00 29.73 C
ATOM 2299 O LYS A 276 40.992 27.206 18.010 1.00 25.10 O
ATOM 2300 CB LYS A 276 43.544 29.051 17.275 1.00 19.19 C
ATOM 2301 CG LYS A 276 43.957 30.496 17.218 1.00 32.11 C
ATOM 2302 CD LYS A 276 44.062 30.852 15.798 1.00 22.43 C
ATOM 2303 CE LYS A 276 44.930 32.067 15.570 1.00 23.18 C
ATOM 2304 NZ LYS A 276 45.454 32.117 14.152 1.00 29.42 N

ATOM	2305	N	PRO A 277	41.892	26.476	16.055	1.00 36.04		N
ATOM	2306	CA	PRO A 277	41.446	25.087	16.170	1.00 35.93		C
ATOM	2307	C	PRO A 277	42.022	24.332	17.363	1.00 29.30		C
ATOM	2308	O	PRO A 277	43.103	24.650	17.885	1.00 30.54		O
ATOM	2309	CB	PRO A 277	41.975	24.453	14.878	1.00 39.65		C
ATOM	2310	CG	PRO A 277	43.249	25.261	14.566	1.00 42.90		C
ATOM	2311	CD	PRO A 277	42.787	26.670	14.892	1.00 37.84		C
ATOM	2312	N	ASP A 278	41.273	23.339	17.809	1.00 22.35		N
ATOM	2313	CA	ASP A 278	41.745	22.501	18.903	1.00 22.16		C
ATOM	2314	C	ASP A 278	42.184	21.189	18.272	1.00 19.66		C
ATOM	2315	O	ASP A 278	41.905	20.917	17.117	1.00 23.49		O
ATOM	2316	CB	ASP A 278	40.636	22.241	19.971	1.00 15.09		C
ATOM	2317	CG	ASP A 278	40.216	23.503	20.702	1.00 22.86		C
ATOM	2318	OD1	ASP A 278	41.113	24.254	21.096	1.00 25.18		O
ATOM	2319	OD2	ASP A 278	38.999	23.787	20.812	1.00 39.55		O
ATOM	2320	N	GLY A 279	42.846	20.355	19.044	1.00 30.65		N
ATOM	2321	CA	GLY A 279	43.229	19.034	18.546	1.00 33.78		C
ATOM	2322	C	GLY A 279	42.115	18.099	18.944	1.00 38.10		C
ATOM	2323	O	GLY A 279	40.963	18.517	19.068	1.00 47.52		O
ATOM	2324	N	THR A 280	42.419	16.839	19.177	1.00 29.44		N
ATOM	2325	CA	THR A 280	41.328	15.990	19.587	1.00 26.68		C
ATOM	2326	C	THR A 280	40.889	16.439	20.972	1.00 23.52		C
ATOM	2327	O	THR A 280	41.670	17.067	21.713	1.00 23.62		O
ATOM	2328	CB	THR A 280	41.695	14.492	19.540	1.00 40.78		C
ATOM	2329	OG1	THR A 280	42.889	14.272	20.296	1.00 25.56		O
ATOM	2330	CG2	THR A 280	41.893	14.054	18.095	1.00 37.71		C
ATOM	2331	N	PRO A 281	39.672	16.063	21.346	1.00 25.54		N
ATOM	2332	CA	PRO A 281	39.129	16.454	22.628	1.00 25.72		C
ATOM	2333	C	PRO A 281	39.776	15.778	23.800	1.00 26.02		C
ATOM	2334	O	PRO A 281	39.752	16.314	24.915	1.00 22.68		O
ATOM	2335	CB	PRO A 281	37.650	15.990	22.559	1.00 28.89		C
ATOM	2336	CG	PRO A 281	37.417	15.540	21.201	1.00 29.39		C
ATOM	2337	CD	PRO A 281	38.761	15.138	20.646	1.00 26.82		C
ATOM	2338	N	ARG A 282	40.281	14.567	23.587	1.00 27.88		N
ATOM	2339	CA	ARG A 282	40.806	13.817	24.720	1.00 34.08		C
ATOM	2340	C	ARG A 282	41.977	12.918	24.384	1.00 27.62		C
ATOM	2341	O	ARG A 282	41.913	12.182	23.425	1.00 23.83		O
ATOM	2342	CB	ARG A 282	39.676	13.017	25.405	1.00 20.89		C
ATOM	2343	CG	ARG A 282	40.035	12.467	26.775	1.00 22.81		C
ATOM	2344	CD	ARG A 282	38.762	11.925	27.442	1.00 26.77		C
ATOM	2345	NE	ARG A 282	38.963	11.345	28.781	1.00 36.48		N
ATOM	2346	CZ	ARG A 282	38.518	10.139	29.164	1.00 37.74		C
ATOM	2347	NH1	ARG A 282	37.813	9.360	28.346	1.00 28.45		N
ATOM	2348	NH2	ARG A 282	38.754	9.700	30.384	1.00 27.25		N
ATOM	2349	N	LYS A 283	43.016	12.963	25.223	1.00 28.91		N

ATOM	2350	CA	LYS A 283	44.217	12.171	25.051	1.00	24.32	C
ATOM	2351	C	LYS A 283	44.796	11.766	26.404	1.00	29.57	C
ATOM	2352	O	LYS A 283	45.262	12.626	27.138	1.00	33.16	O
ATOM	2353	CB	LYS A 283	45.226	13.008	24.287	1.00	21.93	C
ATOM	2354	CG	LYS A 283	46.111	12.251	23.316	1.00	32.38	C
ATOM	2355	CD	LYS A 283	46.526	13.171	22.143	1.00	95.77	C
ATOM	2356	CE	LYS A 283	45.710	12.937	20.836	1.00	100.00	C
ATOM	2357	NZ	LYS A 283	46.418	13.332	19.535	1.00	100.00	N
ATOM	2358	N	LEU A 284	44.747	10.467	26.734	1.00	23.37	N
ATOM	2359	CA	LEU A 284	45.327	9.905	27.997	1.00	16.08	C
ATOM	2360	C	LEU A 284	45.463	8.386	28.047	1.00	20.46	C
ATOM	2361	O	LEU A 284	44.679	7.655	27.446	1.00	25.45	O
ATOM	2362	CB	LEU A 284	44.641	10.387	29.284	1.00	16.30	C
ATOM	2363	CG	LEU A 284	43.334	9.700	29.714	1.00	25.97	C
ATOM	2364	CD1	LEU A 284	42.881	10.089	31.152	1.00	22.11	C
ATOM	2365	CD2	LEU A 284	42.203	9.953	28.693	1.00	23.92	C
ATOM	2366	N	LEU A 285	46.453	7.939	28.820	1.00	18.51	N
ATOM	2367	CA	LEU A 285	46.792	6.527	29.003	1.00	16.77	C
ATOM	2368	C	LEU A 285	45.880	5.865	30.006	1.00	30.75	C
ATOM	2369	O	LEU A 285	45.576	6.439	31.058	1.00	22.02	O
ATOM	2370	CB	LEU A 285	48.229	6.389	29.585	1.00	15.85	C
ATOM	2371	CG	LEU A 285	49.307	6.970	28.672	1.00	21.51	C
ATOM	2372	CD1	LEU A 285	50.703	6.705	29.122	1.00	15.15	C
ATOM	2373	CD2	LEU A 285	49.051	6.368	27.330	1.00	16.94	C
ATOM	2374	N	ASP A 286	45.565	4.599	29.734	1.00	26.62	N
ATOM	2375	CA	ASP A 286	44.945	3.726	30.698	1.00	10.90	C
ATOM	2376	C	ASP A 286	46.128	3.055	31.498	1.00	20.54	C
ATOM	2377	O	ASP A 286	46.991	2.372	30.938	1.00	23.38	O
ATOM	2378	CB	ASP A 286	44.073	2.702	29.970	1.00	14.65	C
ATOM	2379	CG	ASP A 286	43.409	1.699	30.943	1.00	24.60	C
ATOM	2380	OD1	ASP A 286	43.932	1.437	32.083	1.00	24.60	O
ATOM	2381	OD2	ASP A 286	42.316	1.231	30.583	1.00	26.03	O
ATOM	2382	N	VAL A 287	46.230	3.317	32.791	1.00	15.44	N
ATOM	2383	CA	VAL A 287	47.354	2.816	33.556	1.00	15.58	C
ATOM	2384	C	VAL A 287	46.973	1.695	34.521	1.00	16.48	C
ATOM	2385	O	VAL A 287	47.613	1.473	35.572	1.00	16.63	O
ATOM	2386	CB	VAL A 287	48.101	4.006	34.260	1.00	29.84	C
ATOM	2387	CG1	VAL A 287	48.534	5.085	33.224	1.00	18.39	C
ATOM	2388	CG2	VAL A 287	47.173	4.670	35.258	1.00	37.79	C
ATOM	2389	N	THR A 288	45.904	0.992	34.152	1.00	22.27	N
ATOM	2390	CA	THR A 288	45.428	-0.152	34.956	1.00	19.34	C
ATOM	2391	C	THR A 288	46.561	-1.177	35.227	1.00	27.47	C
ATOM	2392	O	THR A 288	46.778	-1.586	36.365	1.00	24.87	O
ATOM	2393	CB	THR A 288	44.288	-0.909	34.244	1.00	22.86	C
ATOM	2394	OG1	THR A 288	43.120	-0.096	34.106	1.00	24.84	O

ATOM	2395	CG2	THR	A	288	43.916	-2.113	35.024	1.00	25.08	C
ATOM	2396	N	ARG	A	289	47.290	-1.585	34.179	1.00	26.08	N
ATOM	2397	CA	ARG	A	289	48.428	-2.506	34.319	1.00	16.92	C
ATOM	2398	C	ARG	A	289	49.405	-2.037	35.408	1.00	22.96	C
ATOM	2399	O	ARG	A	289	49.847	-2.790	36.275	1.00	23.03	O
ATOM	2400	CB	ARG	A	289	49.208	-2.607	32.976	1.00	12.43	C
ATOM	2401	CG	ARG	A	289	48.934	-3.804	32.103	1.00	29.39	C
ATOM	2402	CD	ARG	A	289	50.016	-4.102	31.037	1.00	25.88	C
ATOM	2403	NE	ARG	A	289	49.441	-4.996	30.020	1.00	17.26	N
ATOM	2404	CZ	ARG	A	289	50.053	-5.459	28.930	1.00	38.82	C
ATOM	2405	NH1	ARG	A	289	51.306	-5.153	28.660	1.00	13.51	N
ATOM	2406	NH2	ARG	A	289	49.400	-6.262	28.096	1.00	37.68	N
ATOM	2407	N	LEU	A	290	49.815	-0.786	35.306	1.00	26.60	N
ATOM	2408	CA	LEU	A	290	50.809	-0.254	36.219	1.00	25.42	C
ATOM	2409	C	LEU	A	290	50.324	-0.376	37.656	1.00	24.17	C
ATOM	2410	O	LEU	A	290	51.072	-0.759	38.574	1.00	19.94	O
ATOM	2411	CB	LEU	A	290	51.000	1.219	35.876	1.00	24.66	C
ATOM	2412	CG	LEU	A	290	52.281	2.019	36.066	1.00	24.67	C
ATOM	2413	CD1	LEU	A	290	51.992	3.479	36.504	1.00	29.25	C
ATOM	2414	CD2	LEU	A	290	53.450	1.335	36.788	1.00	15.82	C
ATOM	2415	N	HIS	A	291	49.093	0.075	37.868	1.00	30.10	N
ATOM	2416	CA	HIS	A	291	48.513	0.074	39.212	1.00	34.17	C
ATOM	2417	C	HIS	A	291	48.411	-1.367	39.730	1.00	43.41	C
ATOM	2418	O	HIS	A	291	48.621	-1.654	40.929	1.00	38.81	O
ATOM	2419	CB	HIS	A	291	47.113	0.674	39.143	1.00	28.01	C
ATOM	2420	CG	HIS	A	291	47.097	2.153	38.984	1.00	29.68	C
ATOM	2421	ND1	HIS	A	291	48.242	2.921	39.015	1.00	35.63	N
ATOM	2422	CD2	HIS	A	291	46.068	3.024	38.855	1.00	31.18	C
ATOM	2423	CE1	HIS	A	291	47.926	4.197	38.845	1.00	24.20	C
ATOM	2424	NE2	HIS	A	291	46.612	4.289	38.747	1.00	21.92	N
ATOM	2425	N	GLN	A	292	48.048	-2.260	38.821	1.00	30.71	N
ATOM	2426	CA	GLN	A	292	47.950	-3.654	39.181	1.00	34.82	C
ATOM	2427	C	GLN	A	292	49.287	-4.197	39.622	1.00	36.93	C
ATOM	2428	O	GLN	A	292	49.323	-5.040	40.510	1.00	27.56	O
ATOM	2429	CB	GLN	A	292	47.322	-4.487	38.069	1.00	28.23	C
ATOM	2430	CG	GLN	A	292	45.798	-4.405	38.171	1.00	81.15	C
ATOM	2431	CD	GLN	A	292	45.023	-4.954	36.963	1.00	100.00	C
ATOM	2432	OE1	GLN	A	292	45.597	-5.410	35.951	1.00	99.65	O
ATOM	2433	NE2	GLN	A	292	43.687	-4.895	37.073	1.00	40.86	N
ATOM	2434	N	LEU	A	293	50.375	-3.658	39.058	1.00	31.75	N
ATOM	2435	CA	LEU	A	293	51.750	-4.072	39.383	1.00	22.67	C
ATOM	2436	C	LEU	A	293	52.238	-3.323	40.613	1.00	28.64	C
ATOM	2437	O	LEU	A	293	53.420	-3.377	41.017	1.00	22.27	O
ATOM	2438	CB	LEU	A	293	52.665	-3.769	38.205	1.00	25.57	C
ATOM	2439	CG	LEU	A	293	52.497	-4.703	37.016	1.00	35.11	C

ATOM	2440	CD1	LEU	A	293	53.306	-4.170	35.836	1.00 28.25	C
ATOM	2441	CD2	LEU	A	293	52.965	-6.110	37.439	1.00 47.81	C
ATOM	2442	N	GLY	A	294	51.316	-2.510	41.111	1.00 33.08	N
ATOM	2443	CA	GLY	A	294	51.488	-1.793	42.347	1.00 24.90	C
ATOM	2444	C	GLY	A	294	52.272	-0.512	42.326	1.00 29.31	C
ATOM	2445	O	GLY	A	294	53.070	-0.249	43.223	1.00 25.25	O
ATOM	2446	N	TRP	A	295	52.000	0.347	41.368	1.00 27.83	N
ATOM	2447	CA	TRP	A	295	52.687	1.623	41.385	1.00 19.45	C
ATOM	2448	C	TRP	A	295	51.684	2.731	41.081	1.00 25.79	C
ATOM	2449	O	TRP	A	295	50.765	2.527	40.297	1.00 20.43	O
ATOM	2450	CB	TRP	A	295	53.961	1.614	40.524	1.00 12.85	C
ATOM	2451	CG	TRP	A	295	54.750	2.911	40.618	1.00 23.04	C
ATOM	2452	CD1	TRP	A	295	55.897	3.161	41.368	1.00 23.68	C
ATOM	2453	CD2	TRP	A	295	54.415	4.159	39.979	1.00 20.72	C
ATOM	2454	NE1	TRP	A	295	56.258	4.493	41.244	1.00 18.67	N
ATOM	2455	CE2	TRP	A	295	55.389	5.113	40.373	1.00 20.95	C
ATOM	2456	CE3	TRP	A	295	53.406	4.550	39.102	1.00 21.47	C
ATOM	2457	CZ2	TRP	A	295	55.338	6.439	39.958	1.00 17.58	C
ATOM	2458	CZ3	TRP	A	295	53.403	5.873	38.632	1.00 21.57	C
ATOM	2459	CH2	TRP	A	295	54.368	6.787	39.058	1.00 19.45	C
ATOM	2460	N	TYR	A	296	51.709	3.797	41.884	1.00 25.17	N
ATOM	2461	CA	TYR	A	296	50.720	4.883	41.731	1.00 24.90	C
ATOM	2462	C	TYR	A	296	51.517	6.178	41.857	1.00 30.85	C
ATOM	2463	O	TYR	A	296	52.363	6.272	42.745	1.00 21.27	O
ATOM	2464	CB	TYR	A	296	49.654	4.813	42.840	1.00 25.18	C
ATOM	2465	CG	TYR	A	296	48.685	3.651	42.744	1.00 23.04	C
ATOM	2466	CD1	TYR	A	296	49.078	2.343	43.088	1.00 31.62	C
ATOM	2467	CD2	TYR	A	296	47.380	3.853	42.289	1.00 26.02	C
ATOM	2468	CE1	TYR	A	296	48.203	1.268	42.935	1.00 24.42	C
ATOM	2469	CE2	TYR	A	296	46.493	2.770	42.127	1.00 24.81	C
ATOM	2470	CZ	TYR	A	296	46.902	1.483	42.464	1.00 39.41	C
ATOM	2471	OH	TYR	A	296	45.984	0.434	42.337	1.00 66.19	O
ATOM	2472	N	HIS	A	297	51.324	7.123	40.924	1.00 20.95	N
ATOM	2473	CA	HIS	A	297	52.130	8.343	40.938	1.00 26.86	C
ATOM	2474	C	HIS	A	297	51.947	9.175	42.210	1.00 35.01	C
ATOM	2475	O	HIS	A	297	50.885	9.132	42.874	1.00 26.92	O
ATOM	2476	CB	HIS	A	297	51.819	9.192	39.733	1.00 25.77	C
ATOM	2477	CG	HIS	A	297	50.489	9.842	39.803	1.00 31.16	C
ATOM	2478	ND1	HIS	A	297	49.314	9.145	39.633	1.00 34.21	N
ATOM	2479	CD2	HIS	A	297	50.135	11.094	40.167	1.00 25.83	C
ATOM	2480	CE1	HIS	A	297	48.290	9.972	39.776	1.00 24.14	C
ATOM	2481	NE2	HIS	A	297	48.761	11.164	40.087	1.00 23.35	N
ATOM	2482	N	GLU	A	298	52.983	9.926	42.554	1.00 24.98	N
ATOM	2483	CA	GLU	A	298	52.957	10.683	43.798	1.00 27.65	C
ATOM	2484	C	GLU	A	298	52.831	12.187	43.741	1.00 36.86	C

ATOM	2485	O	GLU A 298	52.433	12.792	44.718	1.00	43.61	O
ATOM	2486	CB	GLU A 298	54.153	10.319	44.686	1.00	22.02	C
ATOM	2487	CG	GLU A 298	54.004	8.943	45.285	1.00	36.42	C
ATOM	2488	CD	GLU A 298	54.999	8.664	46.406	1.00	100.00	C
ATOM	2489	OE1	GLU A 298	56.223	8.561	46.152	1.00	44.79	O
ATOM	2490	OE2	GLU A 298	54.526	8.470	47.547	1.00	100.00	O
ATOM	2491	N	ILE A 299	53.232	12.800	42.639	1.00	23.49	N
ATOM	2492	CA	ILE A 299	53.268	14.244	42.562	1.00	13.25	C
ATOM	2493	C	ILE A 299	52.016	14.848	41.906	1.00	27.05	C
ATOM	2494	O	ILE A 299	51.681	14.530	40.757	1.00	26.73	O
ATOM	2495	CB	ILE A 299	54.586	14.711	41.862	1.00	15.93	C
ATOM	2496	CG1	ILE A 299	55.836	14.183	42.606	1.00	23.83	C
ATOM	2497	CG2	ILE A 299	54.596	16.213	41.541	1.00	17.37	C
ATOM	2498	CD1	ILE A 299	57.232	14.221	41.787	1.00	21.32	C
ATOM	2499	N	SER A 300	51.323	15.716	42.648	1.00	18.55	N
ATOM	2500	CA	SER A 300	50.177	16.449	42.091	1.00	19.58	C
ATOM	2501	C	SER A 300	50.714	17.415	41.042	1.00	17.29	C
ATOM	2502	O	SER A 300	51.824	17.941	41.178	1.00	21.06	O
ATOM	2503	CB	SER A 300	49.542	17.307	43.181	1.00	16.78	C
ATOM	2504	OG	SER A 300	50.548	17.969	43.923	1.00	75.80	O
ATOM	2505	N	LEU A 301	49.870	17.755	40.075	1.00	16.13	N
ATOM	2506	CA	LEU A 301	50.246	18.675	39.014	1.00	17.70	C
ATOM	2507	C	LEU A 301	50.689	19.964	39.646	1.00	20.11	C
ATOM	2508	O	LEU A 301	51.714	20.568	39.303	1.00	20.46	O
ATOM	2509	CB	LEU A 301	48.990	18.981	38.197	1.00	17.92	C
ATOM	2510	CG	LEU A 301	49.182	20.030	37.112	1.00	25.15	C
ATOM	2511	CD1	LEU A 301	50.233	19.552	36.086	1.00	18.82	C
ATOM	2512	CD2	LEU A 301	47.854	20.177	36.436	1.00	25.88	C
ATOM	2513	N	GLU A 302	49.845	20.398	40.554	1.00	27.01	N
ATOM	2514	CA	GLU A 302	50.053	21.636	41.280	1.00	37.72	C
ATOM	2515	C	GLU A 302	51.410	21.618	41.996	1.00	29.99	C
ATOM	2516	O	GLU A 302	52.245	22.514	41.798	1.00	27.15	O
ATOM	2517	CB	GLU A 302	48.899	21.841	42.275	1.00	43.10	C
ATOM	2518	CG	GLU A 302	49.061	23.061	43.174	1.00	90.85	C
ATOM	2519	CD	GLU A 302	48.451	24.324	42.580	1.00	100.00	C
ATOM	2520	OE1	GLU A 302	47.566	24.209	41.706	1.00	100.00	O
ATOM	2521	OE2	GLU A 302	48.808	25.432	43.036	1.00	64.50	O
ATOM	2522	N	ALA A 303	51.646	20.591	42.801	1.00	8.72	N
ATOM	2523	CA	ALA A 303	52.937	20.455	43.459	1.00	15.03	C
ATOM	2524	C	ALA A 303	54.102	20.355	42.450	1.00	19.85	C
ATOM	2525	O	ALA A 303	55.104	21.090	42.553	1.00	22.24	O
ATOM	2526	CB	ALA A 303	52.938	19.258	44.410	1.00	18.97	C
ATOM	2527	N	GLY A 304	53.953	19.472	41.467	1.00	13.05	N
ATOM	2528	CA	GLY A 304	54.970	19.321	40.448	1.00	8.94	C
ATOM	2529	C	GLY A 304	55.239	20.621	39.695	1.00	20.31	C

ATOM 2530 O GLY A 304 56.394 20.900 39.322 1.00 14.30 O
ATOM 2531 N LEU A 305 54.191 21.383 39.361 1.00 10.76 N
ATOM 2532 CA LEU A 305 54.483 22.622 38.611 1.00 20.29 C
ATOM 2533 C LEU A 305 55.281 23.669 39.456 1.00 28.92 C
ATOM 2534 O LEU A 305 56.194 24.385 38.974 1.00 17.69 O
ATOM 2535 CB LEU A 305 53.202 23.245 38.033 1.00 24.03 C
ATOM 2536 CG LEU A 305 52.357 22.647 36.880 1.00 27.66 C
ATOM 2537 CD1 LEU A 305 50.975 23.384 36.789 1.00 13.44 C
ATOM 2538 CD2 LEU A 305 53.079 22.724 35.543 1.00 18.39 C
ATOM 2539 N ALA A 306 54.904 23.757 40.724 1.00 19.94 N
ATOM 2540 CA ALA A 306 55.544 24.660 41.655 1.00 24.79 C
ATOM 2541 C ALA A 306 57.035 24.380 41.743 1.00 27.51 C
ATOM 2542 O ALA A 306 57.852 25.280 41.662 1.00 29.68 O
ATOM 2543 CB ALA A 306 54.937 24.471 43.002 1.00 17.87 C
ATOM 2544 N SER A 307 57.378 23.137 42.011 1.00 18.46 N
ATOM 2545 CA SER A 307 58.793 22.756 42.162 1.00 16.31 C
ATOM 2546 C SER A 307 59.547 22.885 40.832 1.00 22.66 C
ATOM 2547 O SER A 307 60.742 23.212 40.786 1.00 28.47 O
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ATOM   2633  CG  GLN A 316      65.683  31.589  35.231  1.00 80.97           C
ATOM   2634  CD  GLN A 316      65.233  33.036  35.350  1.00 54.58           C
ATOM   2635  OE1 GLN A 316      64.881  33.699  34.367  1.00 46.46           O
ATOM   2636  NE2 GLN A 316      65.257  33.538  36.566  1.00 33.46           N
TER    2637       GLN A 316
CONECT  110  111
CONECT  111  110  112
CONECT  112  111  113  114
CONECT  113  112  118
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CONECT  131  129
CONECT  132  129
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END
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While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. However, it is to be expressly understood that such modifications and adaptations are within the spirit and scope of the present invention, as set forth in the following claims.

What is claimed:

1. A method for producing ascorbic acid or esters thereof in a microorganism, comprising culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase; and recovering said ascorbic acid or esters thereof.

2. A method, as claimed in Claim 1, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

3. A method, as claimed in Claim 1, wherein said genetic modification is a genetic modification to increase the action of an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose.

4. A method, as claimed in Claim 3, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

5. The method of Claim 3, wherein said genetic modification comprises transformation of said microorganism with a recombinant nucleic acid molecule that expresses said epimerase.

6. The method of Claim 5, wherein said epimerase has a tertiary structure that substantially conforms to the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

7. The method of Claim 5, wherein said epimerase has a structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

8. The method of Claim 5, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 1 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

9. The method of Claim 5, wherein said epimerase comprises a substrate binding site having a tertiary structure that substantially conforms to the tertiary structure of the substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

10. The method of Claim 9, wherein said substrate binding site has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a substrate binding site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

11. The method of Claim 5, wherein said epimerase comprises a catalytic site having a tertiary structure that substantially conforms to the tertiary structure of the catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

12. The method of Claim 11, wherein said catalytic site has a tertiary structure with an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a catalytic site of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

13. The method of Claim 11, wherein said catalytic site comprises the amino acid residues serine, tyrosine and lysine.

14. The method of Claim 13, wherein tertiary structure positions of said amino acid residues serine, tyrosine and lysine substantially conform to tertiary structure positions of residues Ser107, Tyr136 and Lys140, respectively, as represented by atomic coordinates in Brookhaven Protein Data Bank Accession Code 1bws.

15. The method of Claim 5, wherein said epimerase binds NADPH.

16. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

17. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 75% of non-Xaa residues in SEQ ID NO:11.

18. The method of Claim 5, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 90% of non-Xaa residues in SEQ ID NO:11.

19. The method of Claim 5, wherein said epimerase comprises an amino acid sequence having at least 4 contiguous amino acid residues that are 100% identical to at least 4 contiguous amino acid residues of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 and SEQ ID NO:10.

20. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence comprising at least about 12 contiguous nucleotides having 100% identity with at least about 12 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9.

21. The method of Claim 5, wherein said epimerase comprises an amino acid sequence having a motif: Gly-Xaa-Xaa-Gly-Xaa-Xaa-Gly.

22. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 15% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

23. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 20% identical to a nucleic acid

sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

24. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that is at least about 25% identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:9, as determined using a Lipman-Pearson method with Lipman-Pearson standard default parameters.

25. The method of Claim 5, wherein said recombinant nucleic acid molecule comprises a nucleic acid sequence that hybridizes under stringent hybridization conditions to a nucleic acid sequence encoding a GDP-4-keto-6-deoxy-D-mannose epimerase/ reductase.

26. The method of Claim 25, wherein said nucleic acid sequence encoding said GDP-4-keto-6-deoxy-D-mannose epimerase/reductase is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:5.

27. The method of Claim 25, wherein said GDP-4-keto-6-deoxy-D-mannose epimerase/reductase comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6.

28. A method, as claimed in Claim 1, wherein said microorganism is selected from the group consisting of bacteria, fungi and microalgae.

29. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant.

30. A method, as claimed in Claim 1, wherein said microorganism is a bacterium.

31. A method, as claimed in Claim 30, wherein said bacterium is selected from the group consisting of *Azotobacter* and *Pseudomonas*.

32. A method, as claimed in Claim 1, wherein said microorganism is a fungus.

33. A method, as claimed in Claim 32, wherein said microorganism is a yeast.

34. A method, as claimed in Claim 33, wherein said yeast is selected from the group consisting of *Saccharomyces* yeast.

35. A method, as claimed in Claim 1, wherein said microorganism is a microalga.

36. A method, as claimed in Claim 35, wherein said microalga is selected from the group consisting of microalgae of the genera *Prototheca* and *Chlorella*.

37. A method, as claimed in Claim 36, wherein said microalga is selected from the genus *Prototheca*.

38. A method, as claimed in Claim 1, wherein said microorganism further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase.

39. A method, as claimed in Claim 38, wherein said genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate, other than GDP-D-mannose:GDP-L-galactose epimerase is a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

40. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 6.0.

41. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 5.5.

42. A method, as claimed in Claim 1, wherein said microorganism is acid-tolerant and said step of culturing is conducted at a pH of less than about 5.0.

43. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that is magnesium (Mg) limited.

44. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that is Mg limited during a cell growth phase.

45. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.5 g/L of Mg during a cell growth phase.

46. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.2 g/L of Mg during a cell growth phase.

47. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises less than about 0.1 g/L of Mg during a cell growth phase.

48. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises a carbon source other than D-mannose.

49. A method, as claimed in Claim 1, wherein said step of culturing is conducted in a fermentation medium that comprises glucose as a carbon source.

50. A microorganism for producing ascorbic acid or esters thereof, wherein said microorganism has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

51. A microorganism, as claimed in Claim 50, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

52. A microorganism, as claimed in Claim 50, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

53. A microorganism, as claimed in Claim 50, wherein said microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

54. A microorganism, as claimed in Claim 50, wherein said microorganism is selected from the group consisting of bacteria, fungi and microalgae.

55. A microorganism, as claimed in Claim 50, wherein said microorganism is a bacterium.

56. A microorganism, as claimed in Claim 55, wherein said bacterium is selected from the group consisting of *Azotobacter* and *Pseudomonas*.

57. A microorganism, as claimed in Claim 50, wherein said microorganism is a fungus.

58. A microorganism, as claimed in Claim 57, wherein said microorganism is a yeast.

59. A microorganism, as claimed in Claim 58, wherein said yeast is selected from the group consisting of *Saccharomyces* yeast.

60. A plant for producing ascorbic acid or esters thereof, wherein said plant has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase, phosphomannose isomerase, phosphomannomutase, GDP-D-mannose pyrophosphorylase, GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

61. A plant, as claimed in Claim 60, wherein said genetic modification is a genetic modification to increase the action of an enzyme selected from the group consisting of GDP-D-mannose:GDP-L-galactose epimerase, GDP-L-galactose phosphorylase, L-galactose-1-P-phosphatase, L-galactose dehydrogenase, and L-galactono-γ-lactone dehydrogenase.

62. A plant, as claimed in Claim 60, wherein said genetic modification is a genetic modification to increase the action of GDP-D-mannose:GDP-L-galactose epimerase.

63. A plant, as claimed in Claim 60, wherein said plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase has a tertiary structure having an average root mean square deviation of less than about 2.5 Å over at least about 25% of Cα positions of the tertiary structure of a GDP-4-keto-6-

deoxy-D-mannose epimerase/reductase represented by atomic coordinates having Brookhaven Protein Data Bank Accession Code 1bws.

64. A plant, as claimed in Claim 60, wherein said plant further comprises a genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase.

65. A plant, as claimed in Claim 60, wherein said genetic modification to decrease the action of an enzyme having GDP-D-mannose as a substrate other than GDP-D-mannose:GDP-L-galactose epimerase is a genetic modification to decrease the action of GDP-D-mannose-dehydrogenase.

66. A plant, as claimed in Claim 60, wherein said plant is a microalga.

67. A plant, as claimed in Claim 66, wherein said plant is selected from the group consisting of microalgae of the genera *Prototheca* and *Chlorella*.

68. A plant, as claimed in Claim 66, wherein said microalga is selected from the genus *Prototheca*.

69. A plant, as claimed in Claim 60, wherein said plant is a higher plant.

70. A plant, as claimed in Claim 60, wherein said plant is a consumable higher plant.

71. A microorganism for producing ascorbic acid or esters thereof, wherein said microorganism has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

72. A plant for producing ascorbic acid or esters thereof, wherein said plant has been genetically modified to express a recombinant nucleic acid molecule encoding an epimerase that catalyzes conversion of GDP-D-mannose to GDP-L-galactose, wherein said epimerase comprises an amino acid sequence that aligns with SEQ ID NO:11 using a CLUSTAL alignment program, wherein amino acid residues in said amino acid sequence align with 100% identity with at least about 50% of non-Xaa residues in SEQ ID NO:11.

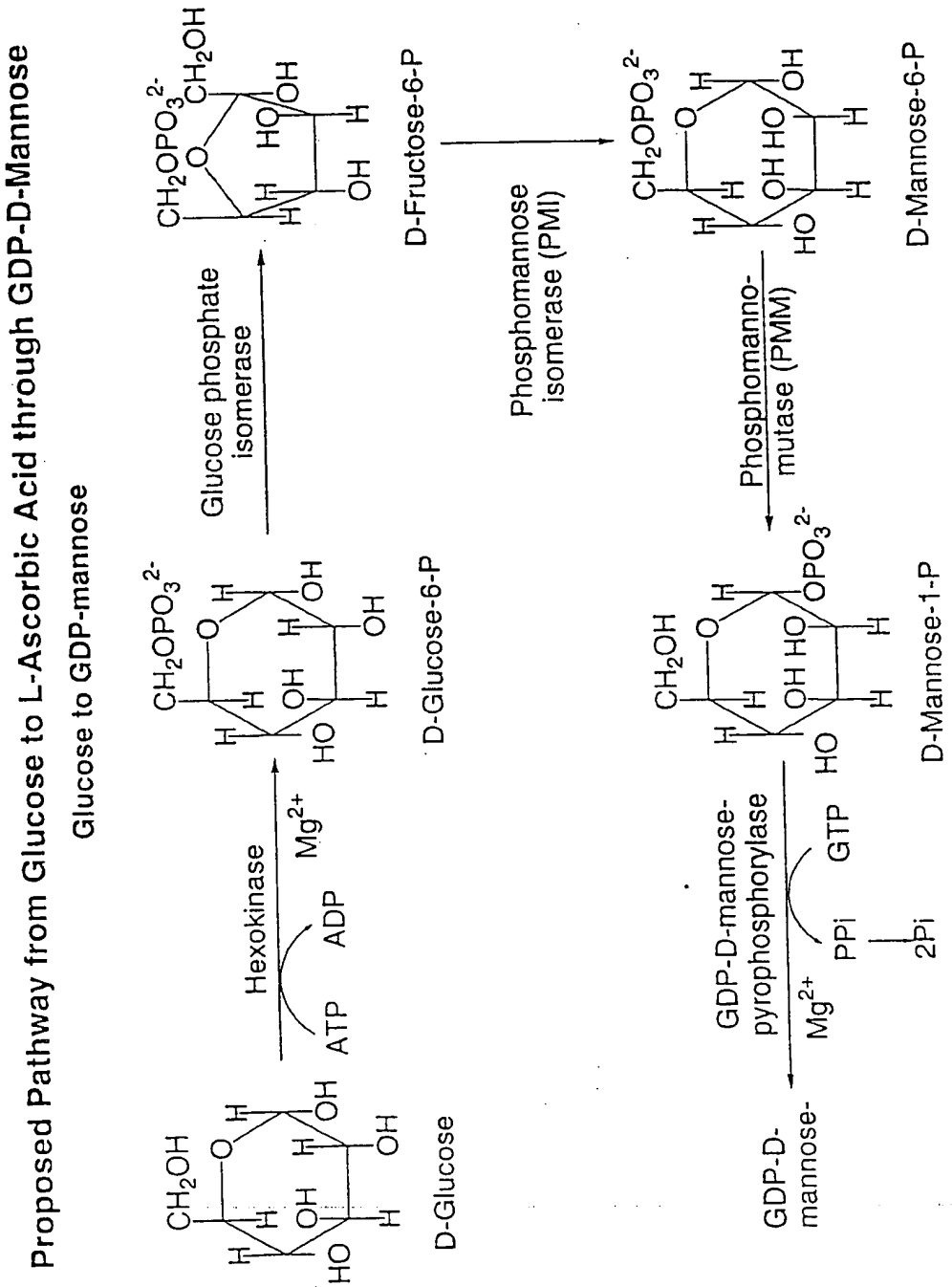
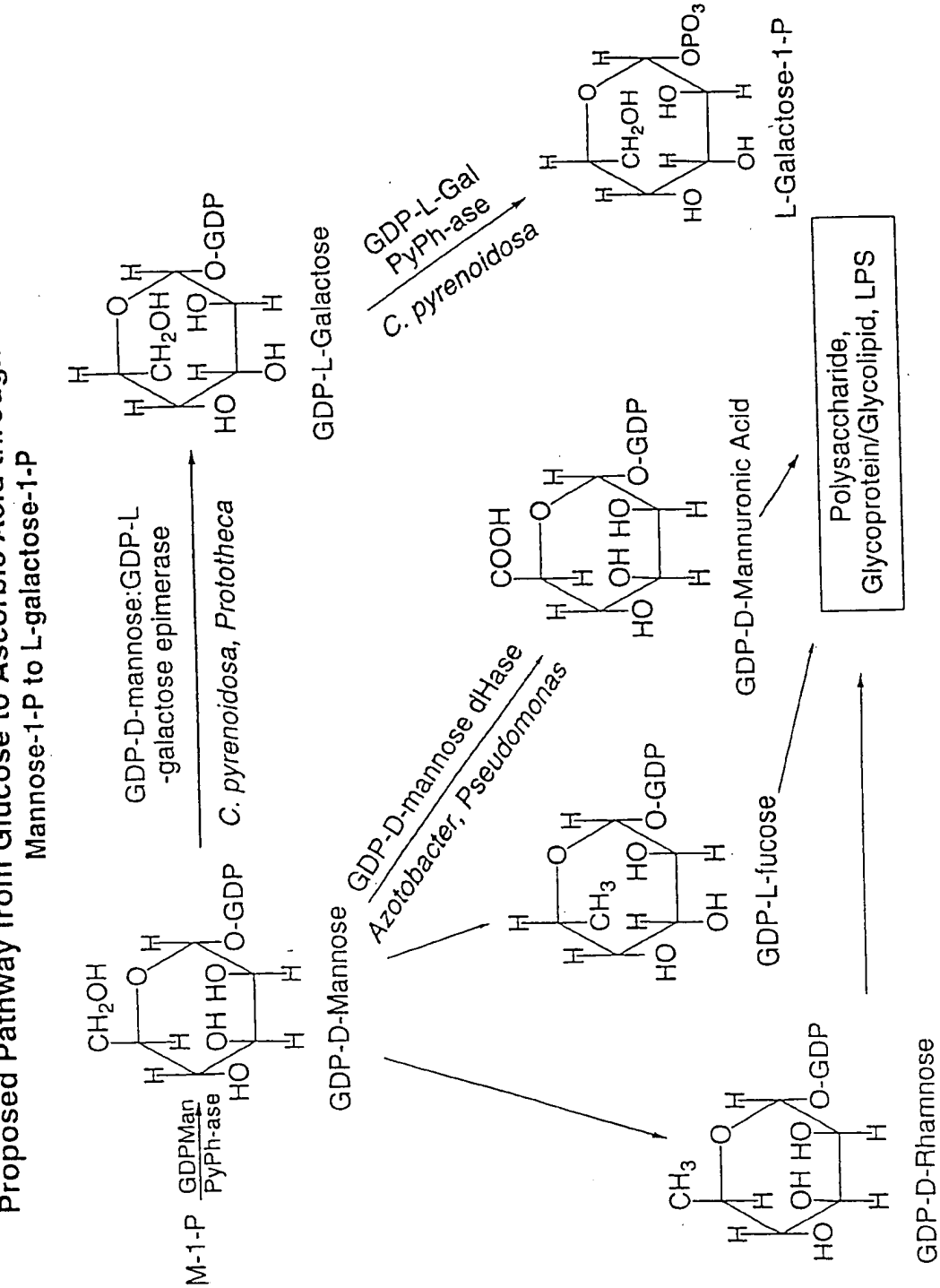

Proposed Pathway from Glucose to L-Ascorbic Acid through GDP-D-Mannose
Glucose to GDP-mannose
D-Glucose
Hexokinase
ATP
ADP
Mg^{2+}
D-Glucose-6-P
Glucose phosphate isomerase
D-Fructose-6-P
Phosphomannose isomerase (PMI)
D-Mannose-6-P
Phosphomanno-mutase (PMM)
D-Mannose-1-P
GDP-D-mannose-pyrophosphorylase
GTP
PPi
2Pi
Mg^{2+}
GDP-D-mannose-

FIG. 1A


Proposed Pathway from Glucose to Ascorbic Acid through GDP-D-Mannose
Mannose-1-P to L-galactose-1-P
M-1-P
GDPMan
PyPh-ase
GDP-D-Mannose
GDP-D-mannose:GDP-L
-galactose epimerase
C. pyrenoidosa, Prototheca
GDP-L-Galactose
GDP-L-Gal
PyPh-ase
C. pyrenoidosa
L-Galactose-1-P
GDP-D-mannose dHase
Azotobacter, Pseudomonas
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GDP-L-fucose
Polysaccharide,
Glycoprotein/Glycolipid, LPS
GDP-D-Rhamnose

FIG. 1B

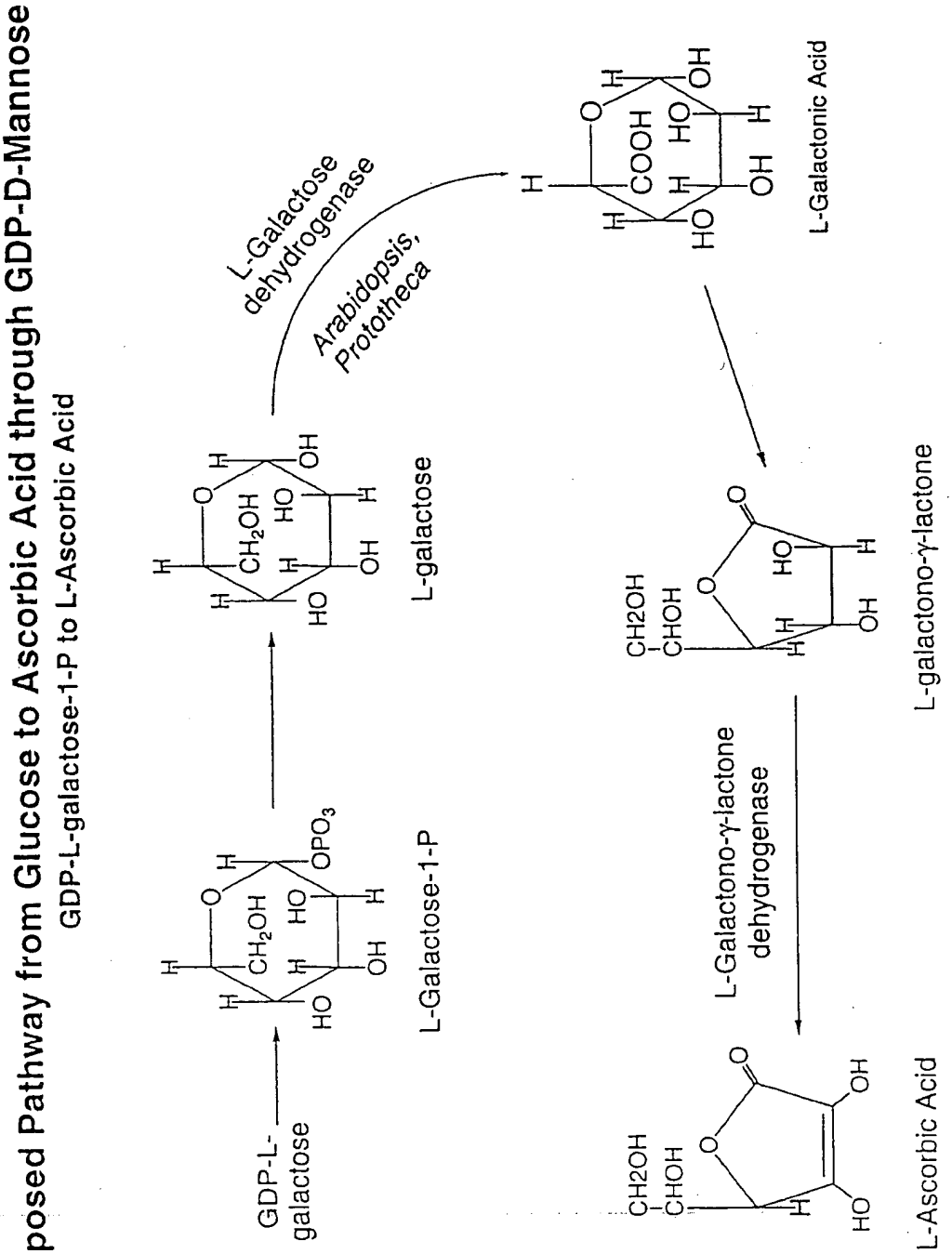
Proposed Pathway from Glucose to Ascorbic Acid through GDP-D-Mannose
GDP-L-galactose-1-P to L-Ascorbic Acid
GDP-L-galactose
L-Galactose-1-P
L-galactose
L-Galactose dehydrogenase
Arabidopsis, Prototheca
L-Galactonic Acid
L-Galactono-γ-lactone dehydrogenase
L-galactono-γ-lactone
L-Ascorbic Acid

FIG. 1C

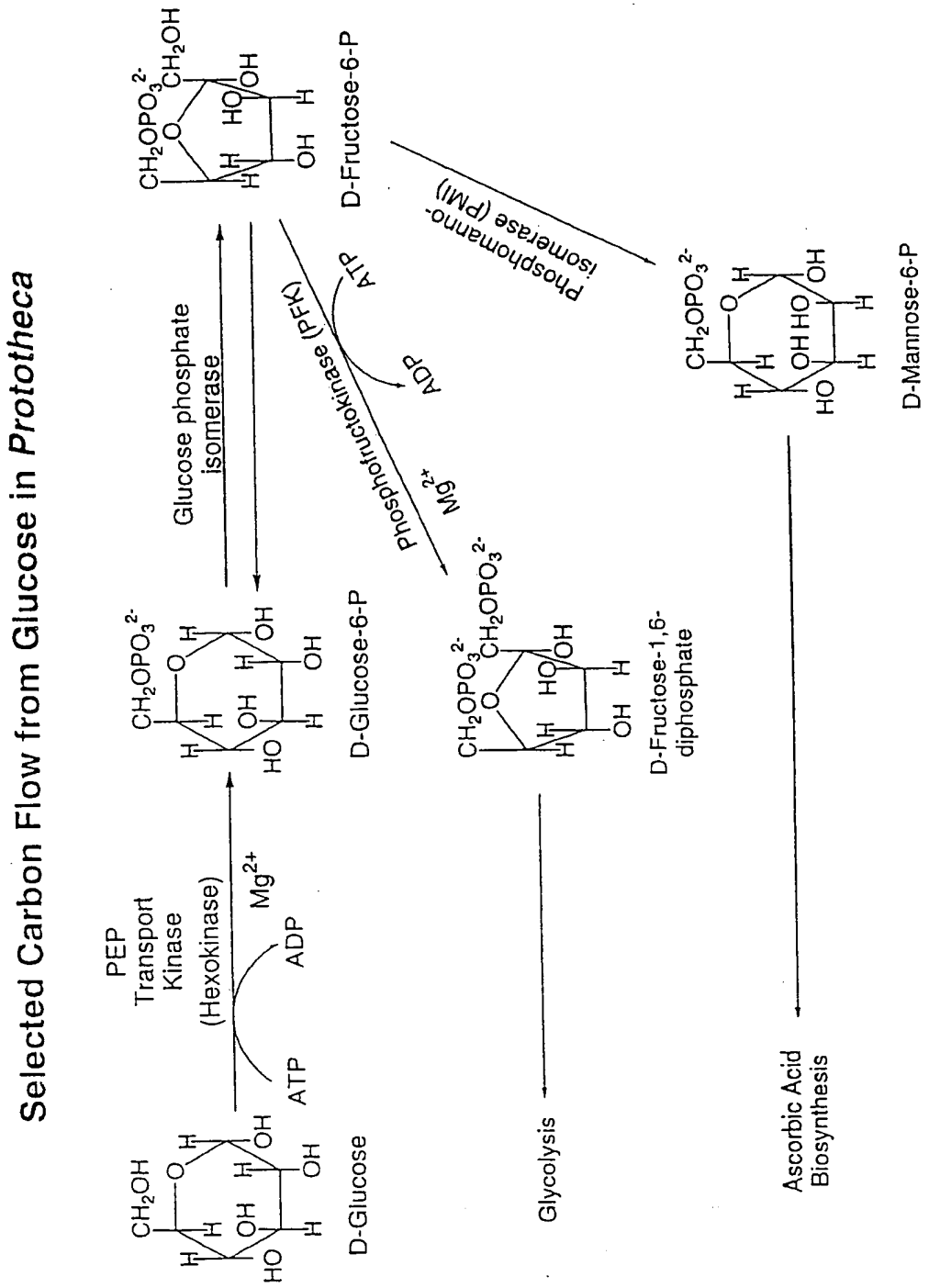
Selected Carbon Flow from Glucose in *Prototheca*
D-Glucose
PEP Transport Kinase (Hexokinase)
ATP
ADP
Mg^{2+}
D-Glucose-6-P
Glucose phosphate isomerase
D-Fructose-6-P
Phosphofructokinase (PFK)
ATP
ADP
Mg^{2+}
D-Fructose-1,6-diphosphate
Glycolysis
Phosphomanno-isomerase (PMI)
D-Mannose-6-P
Ascorbic Acid Biosynthesis

FIG. 2A

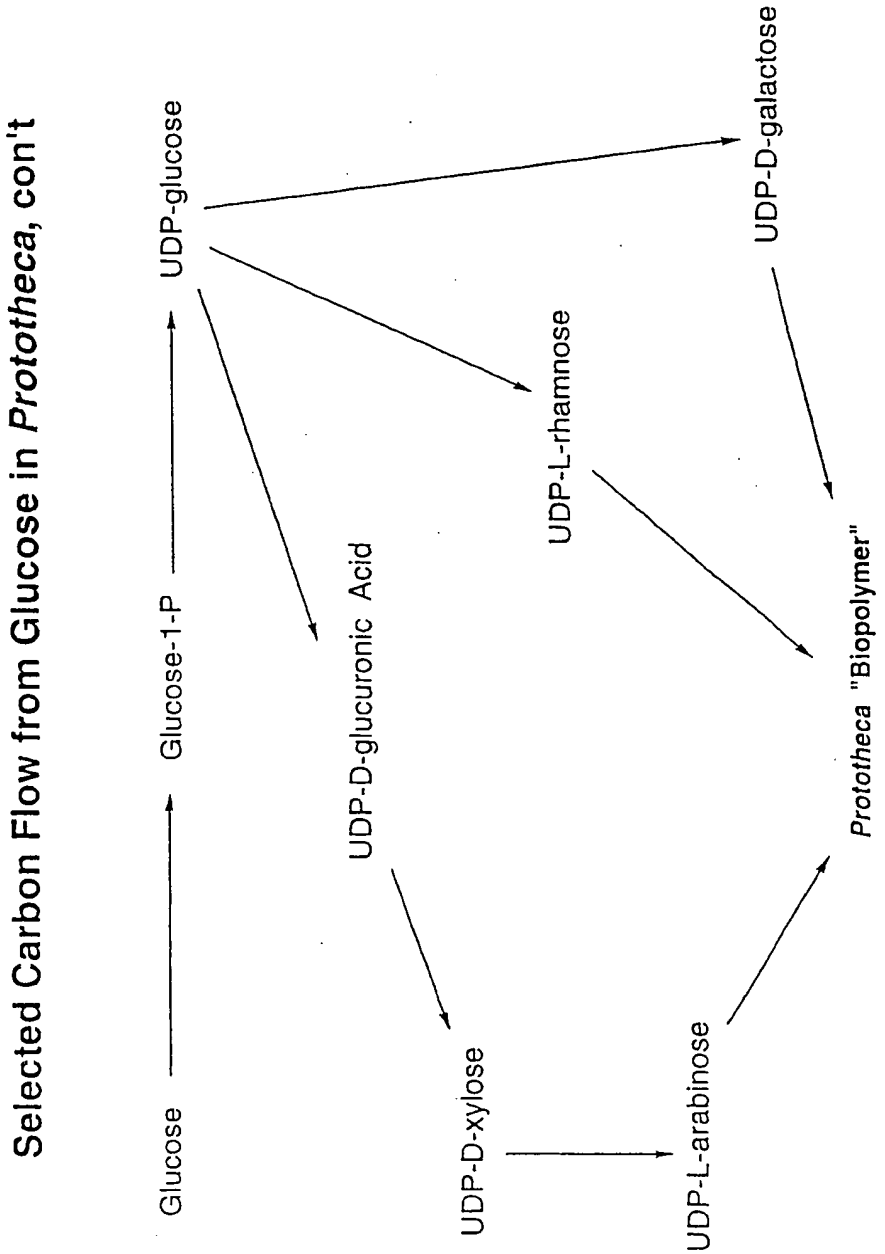
Selected Carbon Flow from Glucose in *Prototheca*, con't
Glucose
Glucose-1-P
UDP-glucose
UDP-D-glucuronic Acid
UDP-L-rhamnose
UDP-D-galactose
UDP-D-xylose
UDP-L-arabinose
Prototheca "Biopolymer"

FIG. 2B

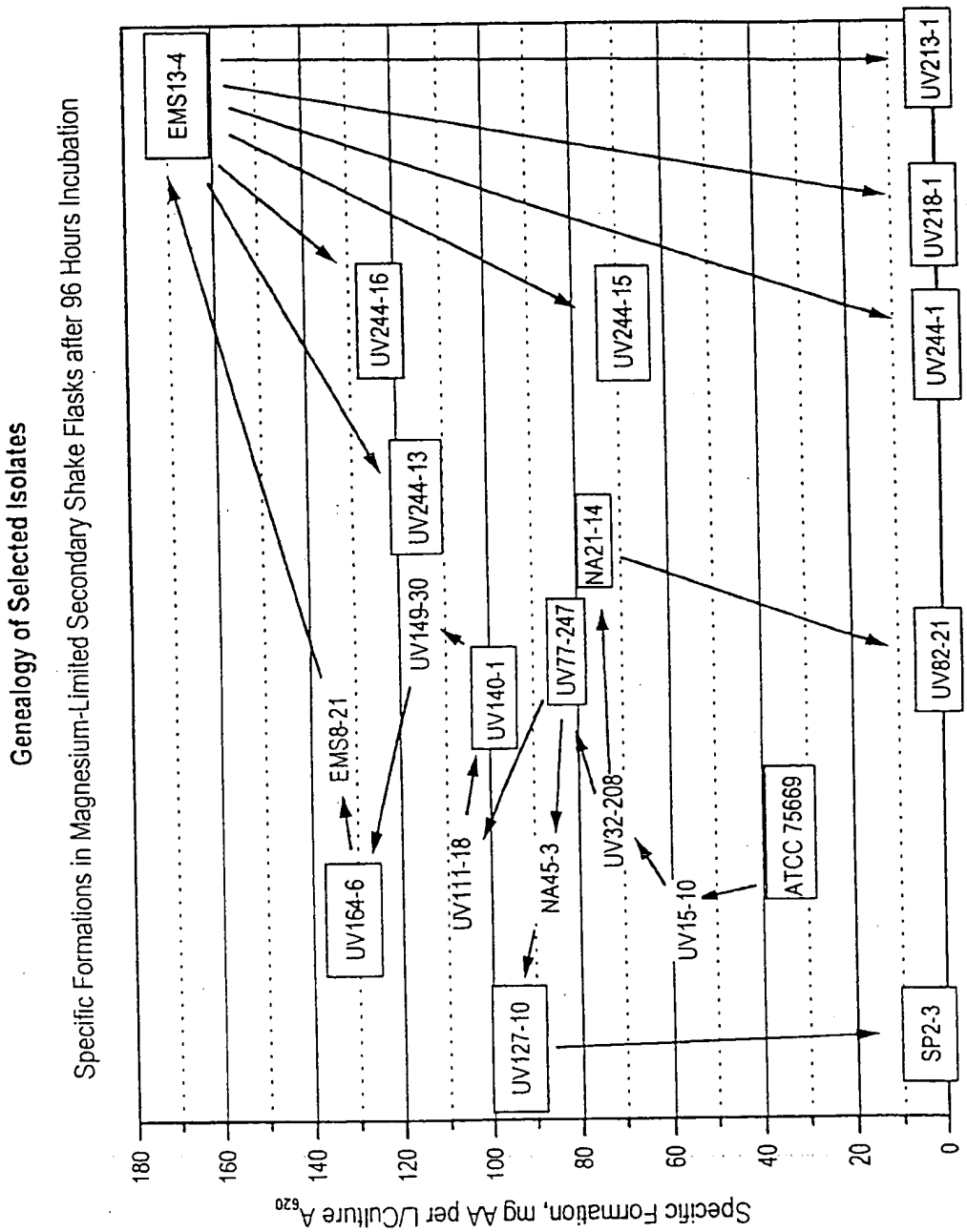
Genealogy of Selected Isolates
Specific Formations in Magnesium-Limited Secondary Shake Flasks after 96 Hours Incubation
Specific Formation, mg AA per L/Culture A_{620}
180
160
140
120
100
80
60
40
20
0
EMS13-4
UV164-6
EMS8-21
UV244-16
UV244-13
UV149-30
UV111-18
UV140-1
UV127-10
UV77-247
NA45-3
NA21-14
UV32-208
UV15-10
ATCC 75669
UV244-15
SP2-3
UV82-21
UV244-1
UV218-1
UV213-1

FIG. 3

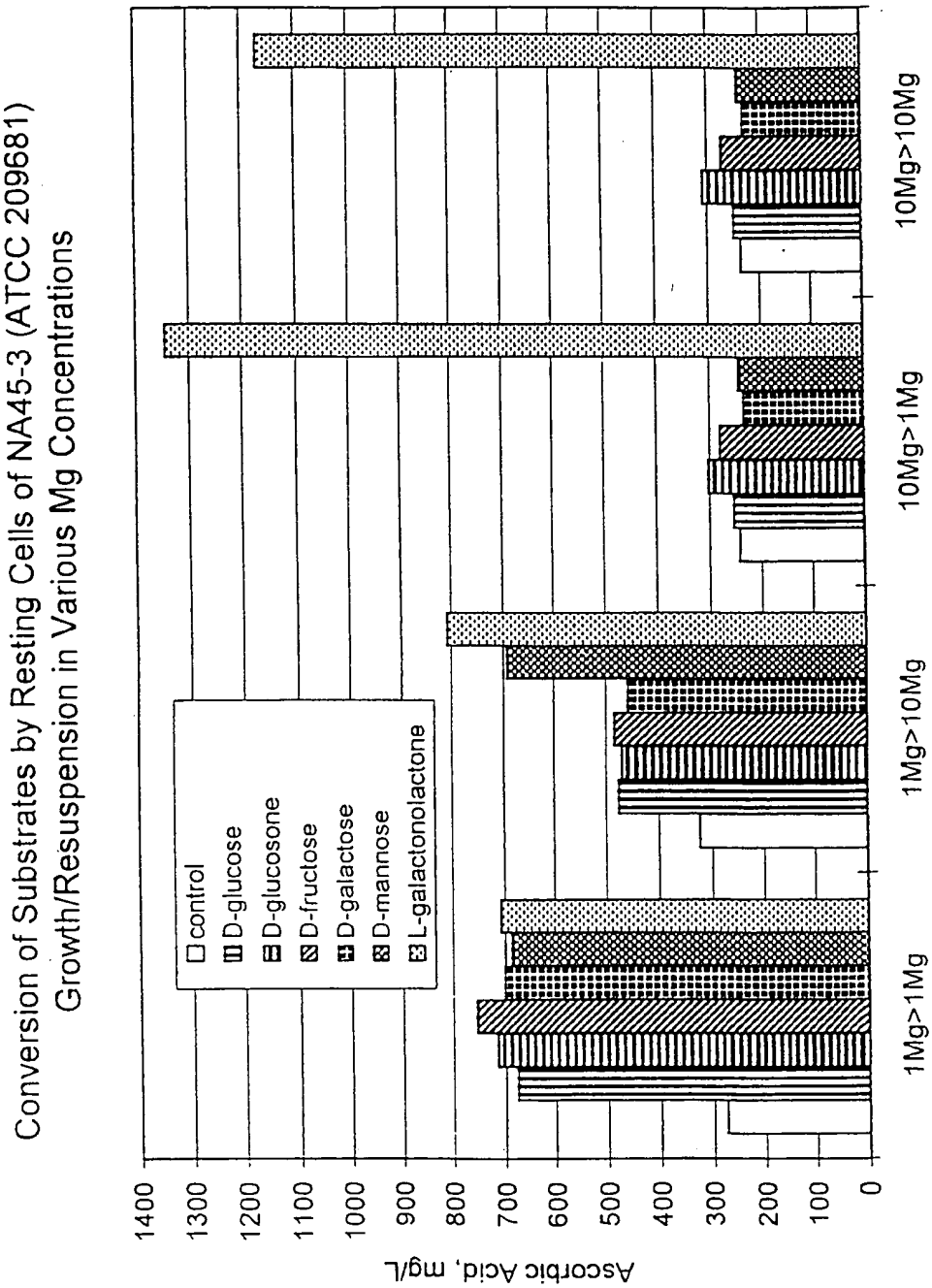
Conversion of Substrates by Resting Cells of NA45-3 (ATCC 209681)
Growth/Resuspension in Various Mg Concentrations
control
D-glucose
D-glucosone
D-fructose
D-galactose
D-mannose
L-galactonolactone
Ascorbic Acid, mg/L
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1Mg>1Mg
1Mg>10Mg
10Mg>1Mg
10Mg>10Mg
Growth>Resuspnsion [Mg]

Fig. 4

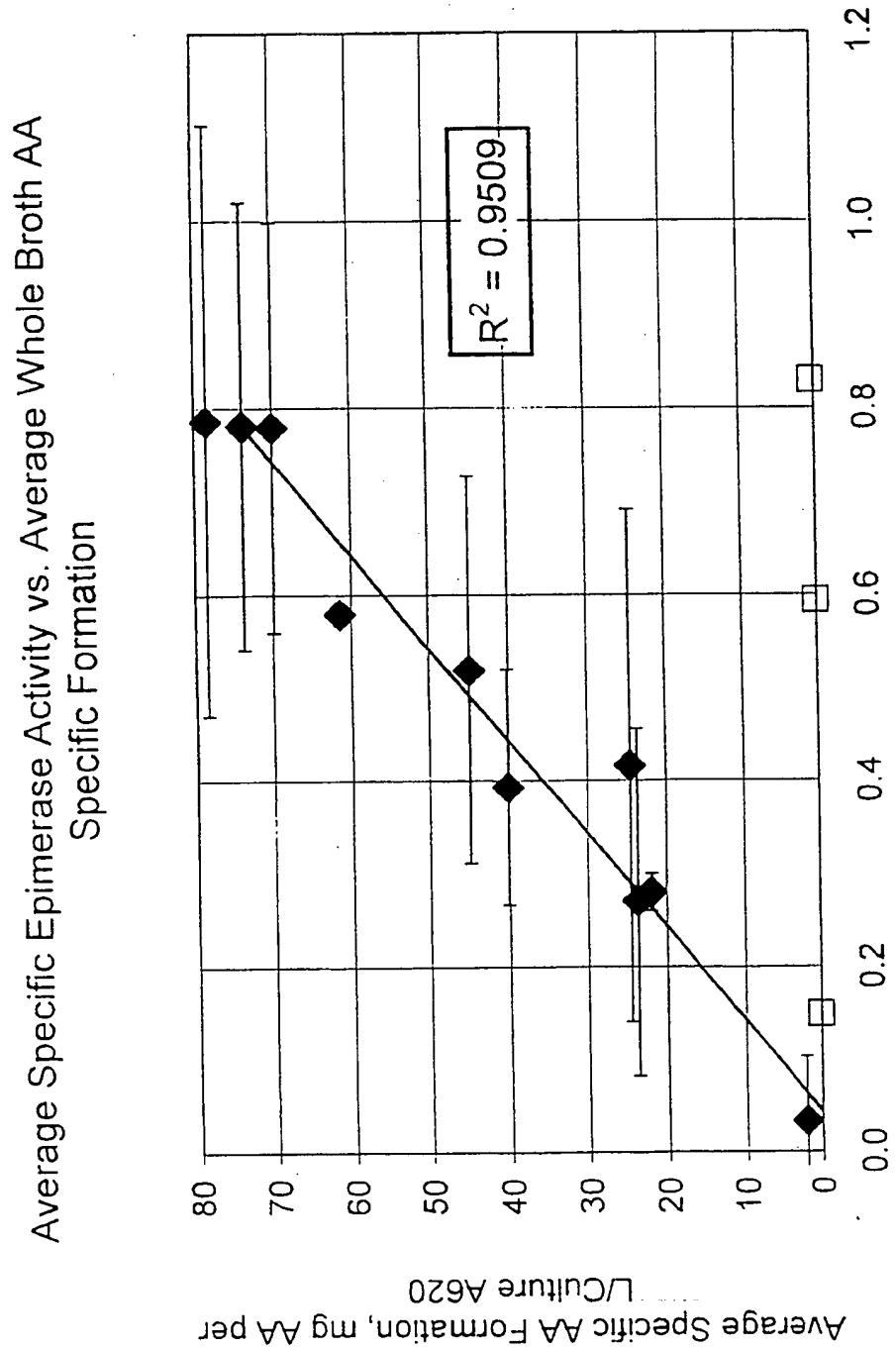
Average Specific Epimerase Activity vs. Average Whole Broth AA Specific Formation
R^2 = 0.9509
Average Specific AA Formation, mg AA per L/Culture A620
Average Specific Epimerase Activity, mU
0
10
20
30
40
50
60
70
80
0.0
0.2
0.4
0.6
0.8
1.0
1.2

Fig. 5

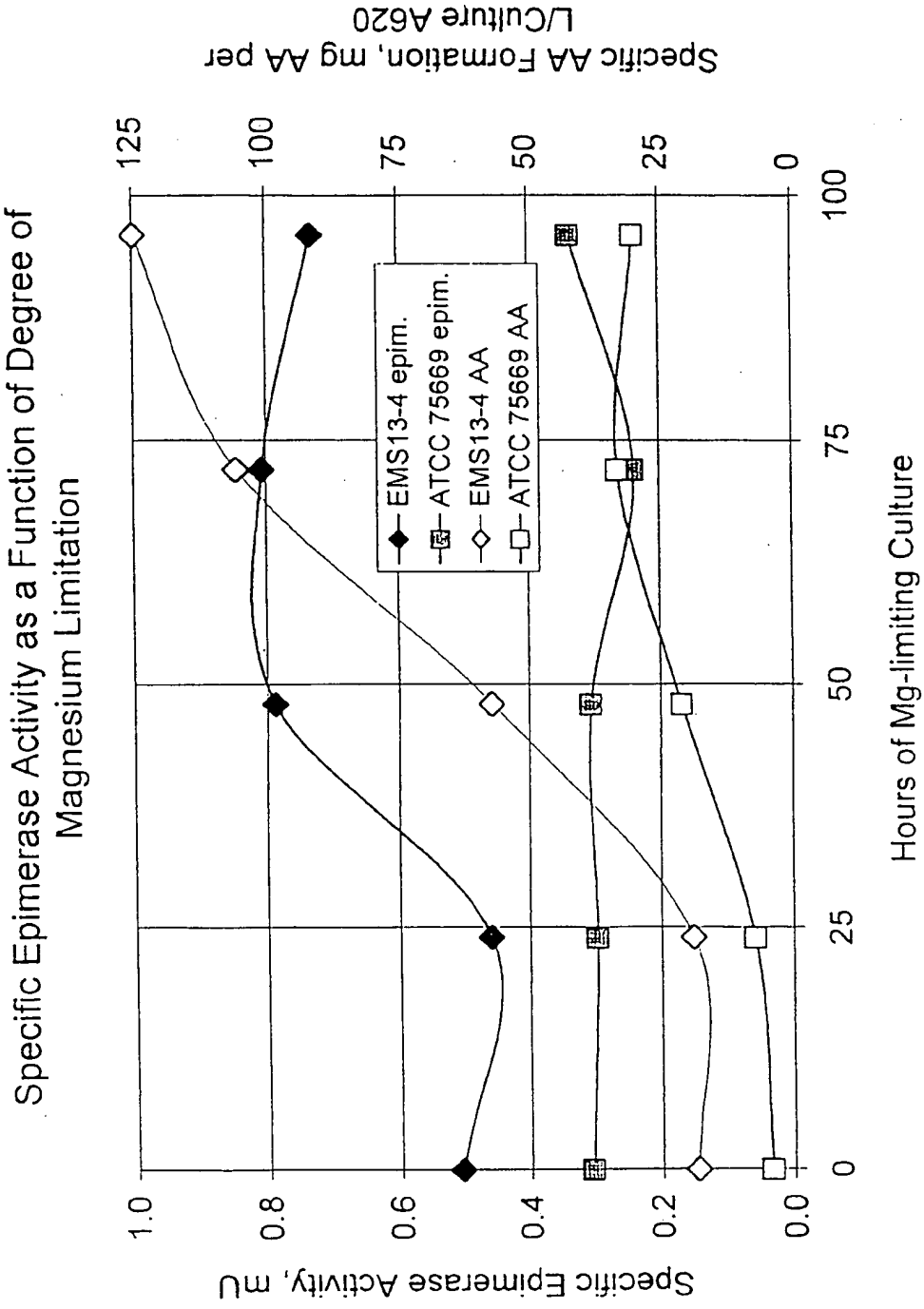
Specific Epimerase Activity as a Function of Degree of Magnesium Limitation
Specific Epimerase Activity, mU
1.0
0.8
0.6
0.4
0.2
0.0
Specific AA Formation, mg AA per L/Culture A620
125
100
75
50
25
0
Hours of Mg-limiting Culture
0
25
50
75
100
EMS13-4 epim.
ATCC 75669 epim.
EMS13-4 AA
ATCC 75669 AA

Fig. 6

Proposed Mechanism for the Conversion of GDP-D-mannose to GDP-L-galactose in *Chlorella pyrenoidosa* (Barber)

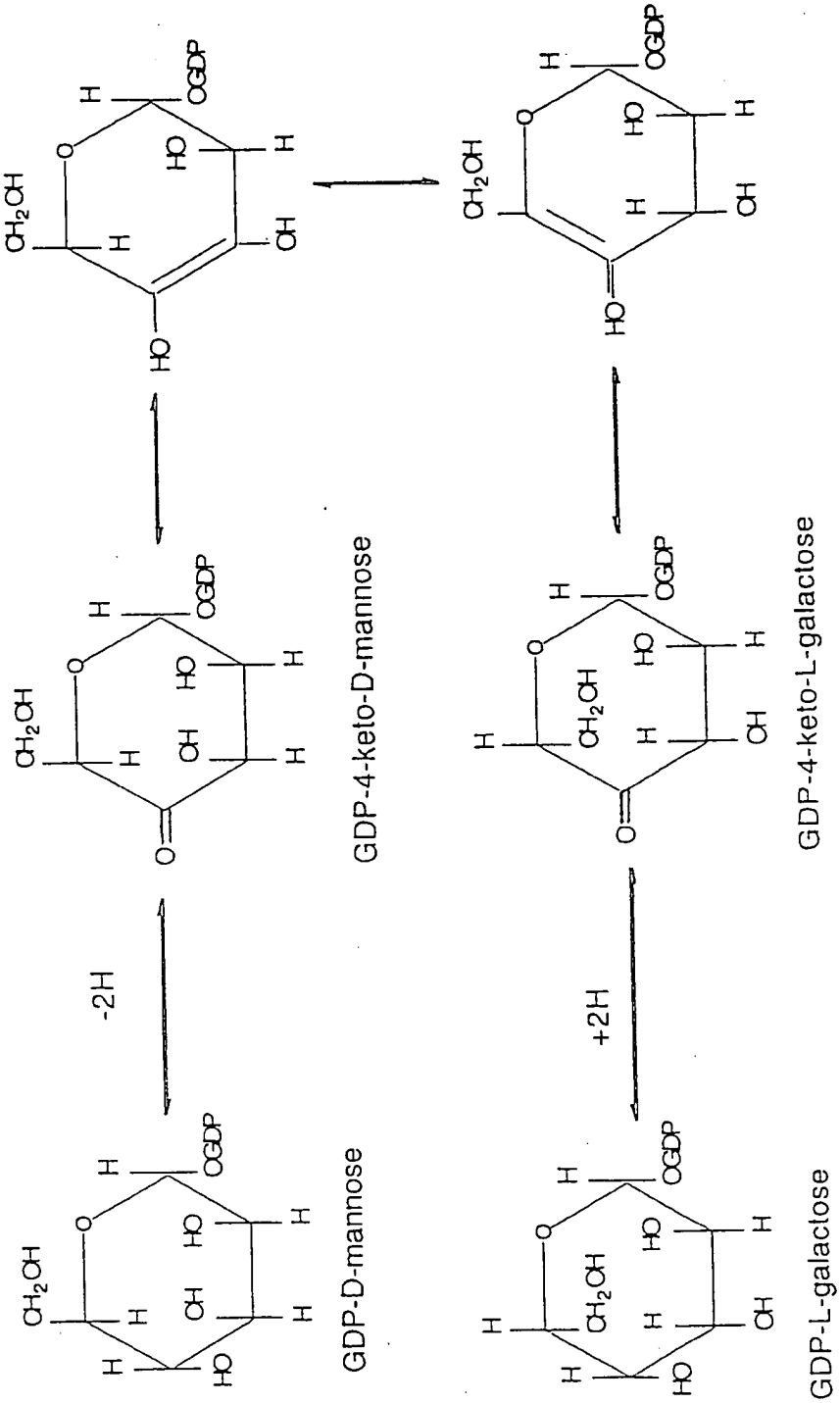

Fig. 7

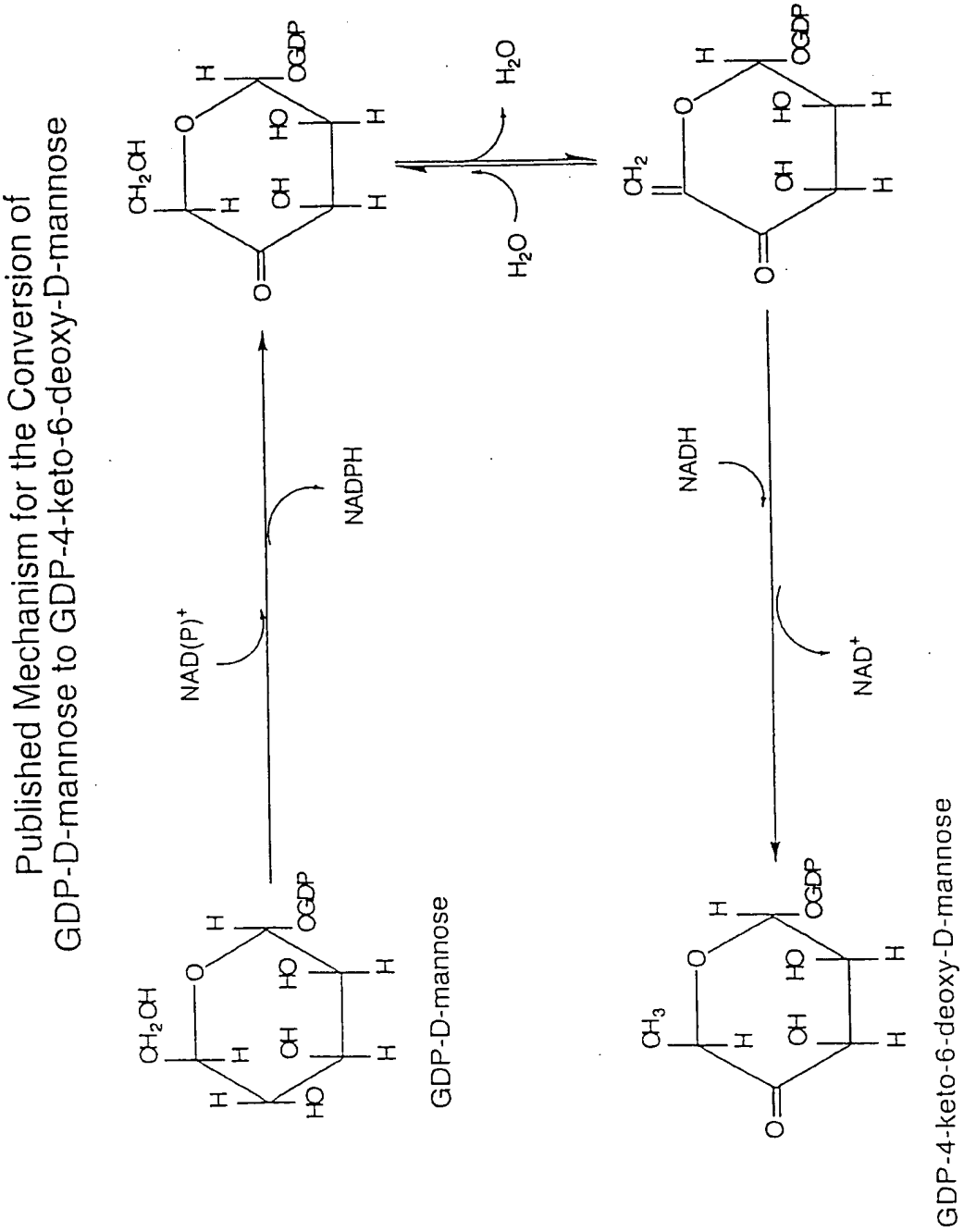
Published Mechanism for the Conversion of
GDP-D-mannose to GDP-4-keto-6-deoxy-D-mannose
NAD(P)+
NADPH
H2O
H2O
NADH
NAD+
GDP-D-mannose
GDP-4-keto-6-deoxy-D-mannose

Fig. 8A

Published Mechanism for the Conversion of
GDP-4-keto-6-deoxy-D-mannose to GDP-L-fucose

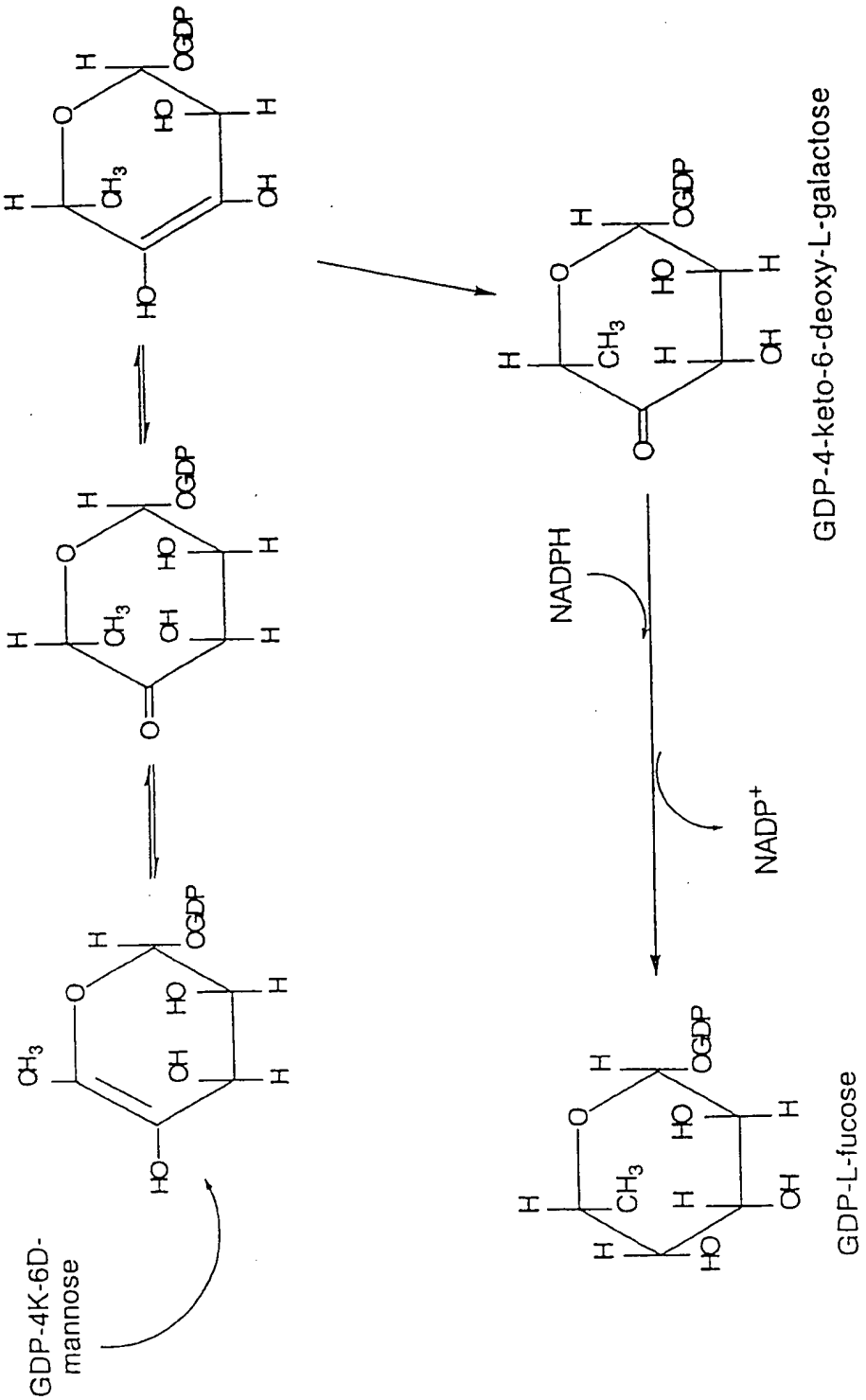

Fig. 8B

SEQUENCE LISTING

<110> Berry, Alan
Running, Jeffrey A.
Severson, David K.
Burlingame, Richard P.

<120> "VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS"

<130> 3161-24-PCT

<140> not yet assigned
<141> 1999-05-25

<150> 60/125,073
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Lys Ser Ala Lys Ile Phe Val Ala Gly His Arg Gly Leu Val Gly Ser
5 10 15

gcc att gtc cgc aag ctt cag gaa caa ggt ttc acc aat ctc gtt ctt 153
Ala Ile Val Arg Lys Leu Gln Glu Gln Gly Phe Thr Asn Leu Val Leu
20 25 30 35

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Lys Thr His Ala Glu Leu Asp Leu Thr Arg Gln Ala Asp Val Glu Ser
                 40                  45                  50

ttc ttt tct caa gag aag cca gtt tat gta atc cta gca gca gct aaa   249
Phe Phe Ser Gln Glu Lys Pro Val Tyr Val Ile Leu Ala Ala Ala Lys
            55                  60                  65

gtt ggt ggt att cac gct aac aac acc tat cct gct gat ttc att ggt   297
Val Gly Gly Ile His Ala Asn Asn Thr Tyr Pro Ala Asp Phe Ile Gly
        70                  75                  80

gtc aat ctc cag att cag acc aat gtg atc cac tct gca tat gag cac   345
Val Asn Leu Gln Ile Gln Thr Asn Val Ile His Ser Ala Tyr Glu His
    85                  90                  95

ggt gtg aag aag ctt ctc ttc ctt gga tca tcc tgc att tac cct aaa   393
Gly Val Lys Lys Leu Leu Phe Leu Gly Ser Ser Cys Ile Tyr Pro Lys
100                 105                 110                 115

ttt gct cct cag cca att cct gag tct gct ttg tta aca gca tcg ctt   441
Phe Ala Pro Gln Pro Ile Pro Glu Ser Ala Leu Leu Thr Ala Ser Leu
                120                 125                 130

gaa cca act aat gag tgg tat gct att gct aag atc gct ggg att aag   489
Glu Pro Thr Asn Glu Trp Tyr Ala Ile Ala Lys Ile Ala Gly Ile Lys
            135                 140                 145

act tgt cag gct tat agg att cag cac gga tgg gat gca atc tct ggc   537
Thr Cys Gln Ala Tyr Arg Ile Gln His Gly Trp Asp Ala Ile Ser Gly
        150                 155                 160

atg cct act aat ctc tat ggt cct aat gac aat ttc cac ccg gag tct   585
Met Pro Thr Asn Leu Tyr Gly Pro Asn Asp Asn Phe His Pro Glu Ser
    165                 170                 175

cat gtg ctt cct gct ctt atg agg agg ttc cac gag gcg aaa gtg aat   633
His Val Leu Pro Ala Leu Met Arg Arg Phe His Glu Ala Lys Val Asn
180                 185                 190                 195

tgg agc gga gga agt tgt ggt gtg ggg tac aag gta gtc ccg ttg gaa   681
Trp Ser Gly Gly Ser Cys Gly Val Gly Tyr Lys Val Val Pro Leu Glu
                200                 205                 210

ggg aag ttc ttg cat gtt gat gat ttg gct gat gct tgt gtt ttc ttg   729
Gly Lys Phe Leu His Val Asp Asp Leu Ala Asp Ala Cys Val Phe Leu
            215                 220                 225
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ctg gat cgg ata cag cgg ggg ttg gag cat gtt aac att gga agt ggt   777
Leu Asp Arg Ile Gln Arg Gly Leu Glu His Val Asn Ile Gly Ser Gly
        230                 235                 240

caa gaa gtg act att aga gag ttg gct gag ttg gtg aaa gag gtt gtt   825
Gln Glu Val Thr Ile Arg Glu Leu Ala Glu Leu Val Lys Glu Val Val
    245                 250                 255

ggt ttt gaa ggg aag ctt gga tgg gat tgc act aag cca gat ggc aca   873
Gly Phe Glu Gly Lys Leu Gly Trp Asp Cys Thr Lys Pro Asp Gly Thr
260                 265                 270                 275

ccg agg aaa ctt atg gac agc tca aag ctc gcg tct ttg ggt tgg aca   921
Pro Arg Lys Leu Met Asp Ser Ser Lys Leu Ala Ser Leu Gly Trp Thr
                280                 285                 290

cct aag gtt tct ctt aga gat ggt ctg agc caa act tat gat tgg tat   969
Pro Lys Val Ser Leu Arg Asp Gly Leu Ser Gln Thr Tyr Asp Trp Tyr
            295                 300                 305

ttg aag aat gtt tgc aac cga taa gttaatggtt tctcttctca tatatacaca  1023
Leu Lys Asn Val Cys Asn Arg
        310                 315

actattgagt ctcaggtaaa tcagcttatc accacattgt gatttaaacc tttctttgag 1083

attcgagaat tgctttttt tttatcaaaa ttgattcatt tagagataag acttgcttct 1143

ttatacaaca ttgtctgagg aattttaatt ttggatctcc gagtatggtc tattattagc 1203

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cccaatgggc ttacacaagc cttgttaaag atagcgtgaa caaaacgcgg ctcactagcc 1383

ctaacctgtc tctctttcgc ttaccttctt cttcgtcttc gttggctcag tcacttgact 1443

tcacggcccg ctcaagctct gacacgaaac tcatttcaaa ttaatttaat aaaaccttaa 1503

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gttgtgagcc aacccgttcg                                            1583
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            20                  25                  30

Leu Val Leu Lys Thr His Ala Glu Leu Asp Leu Thr Arg Gln Ala Asp
        35                  40                  45

Val Glu Ser Phe Phe Ser Gln Glu Lys Pro Val Tyr Val Ile Leu Ala
    50                  55                  60

Ala Ala Lys Val Gly Gly Ile His Ala Asn Asn Thr Tyr Pro Ala Asp
65                  70                  75                  80

Phe Ile Gly Val Asn Leu Gln Ile Gln Thr Asn Val Ile His Ser Ala
                85                  90                  95

Tyr Glu His Gly Val Lys Lys Leu Leu Phe Leu Gly Ser Ser Cys Ile
            100                 105                 110

Tyr Pro Lys Phe Ala Pro Gln Pro Ile Pro Glu Ser Ala Leu Leu Thr
        115                 120                 125

Ala Ser Leu Glu Pro Thr Asn Glu Trp Tyr Ala Ile Ala Lys Ile Ala
    130                 135                 140

Gly Ile Lys Thr Cys Gln Ala Tyr Arg Ile Gln His Gly Trp Asp Ala
145                 150                 155                 160

Ile Ser Gly Met Pro Thr Asn Leu Tyr Gly Pro Asn Asp Asn Phe His
                165                 170                 175

Pro Glu Ser His Val Leu Pro Ala Leu Met Arg Arg Phe His Glu Ala
            180                 185                 190

Lys Val Asn Trp Ser Gly Gly Ser Cys Gly Val Gly Tyr Lys Val Val
        195                 200                 205

Pro Leu Glu Gly Lys Phe Leu His Val Asp Asp Leu Ala Asp Ala Cys
    210                 215                 220

Val Phe Leu Leu Asp Arg Ile Gln Arg Gly Leu Glu His Val Asn Ile
225                 230                 235                 240
```

```
Gly Ser Gly Gln Glu Val Thr Ile Arg Glu Leu Ala Glu Leu Val Lys
                245                 250                 255

Glu Val Val Gly Phe Glu Gly Lys Leu Gly Trp Asp Cys Thr Lys Pro
            260                 265                 270

Asp Gly Thr Pro Arg Lys Leu Met Asp Ser Ser Lys Leu Ala Ser Leu
        275                 280                 285

Gly Trp Thr Pro Lys Val Ser Leu Arg Asp Gly Leu Ser Gln Thr Tyr
    290                 295                 300

Asp Trp Tyr Leu Lys Asn Val Cys Asn Arg
305                 310
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Met Ser Lys Gln Arg Val Phe Ile Ala Gly His Arg Gly Met Val Gly
  1               5                  10                  15

tcc gcc atc agg cgg cag ctc gaa cag cgc ggt gat gtg gaa ctg gta   96
Ser Ala Ile Arg Arg Gln Leu Glu Gln Arg Gly Asp Val Glu Leu Val
             20                  25                  30

tta cgc acc cgc gac gag ctg aac ctg ctg gac agc cgc gcc gtg cat   144
Leu Arg Thr Arg Asp Glu Leu Asn Leu Leu Asp Ser Arg Ala Val His
         35                  40                  45

gat ttc ttt gcc agc gaa cgt att gac cag gtc tat ctg gcg gcg gcg   192
Asp Phe Phe Ala Ser Glu Arg Ile Asp Gln Val Tyr Leu Ala Ala Ala
     50                  55                  60

aaa gtg ggc ggc att gtt gcc aac aac acc tat ccg gcg gat ttc atc   240
Lys Val Gly Gly Ile Val Ala Asn Asn Thr Tyr Pro Ala Asp Phe Ile
 65                  70                  75                  80

tac cag aac atg atg att gag agc aac atc att cac gcc gcg cat cag   288
```

```
Tyr Gln Asn Met Met Ile Glu Ser Asn Ile Ile His Ala Ala His Gln
                85                  90                  95

aac gac gtg aac aaa ctg ctg ttt ctc gga tcg tcc tgc atc tac ccg   336
Asn Asp Val Asn Lys Leu Leu Phe Leu Gly Ser Ser Cys Ile Tyr Pro
            100                 105                 110

aaa ctg gca aaa cag ccg atg gca gaa agc gag ttg ttg cag ggc acg   384
Lys Leu Ala Lys Gln Pro Met Ala Glu Ser Glu Leu Leu Gln Gly Thr
        115                 120                 125

ctg gag ccg act aac gag cct tat gct att gcc aaa atc gcc ggg atc   432
Leu Glu Pro Thr Asn Glu Pro Tyr Ala Ile Ala Lys Ile Ala Gly Ile
    130                 135                 140

aaa ctg tgc gaa tca tac aac cgc cag tac gga cgc gat tac cgc tca   480
Lys Leu Cys Glu Ser Tyr Asn Arg Gln Tyr Gly Arg Asp Tyr Arg Ser
145                 150                 155                 160

gtc atg ccg acc aac ctg tac ggg cca cac gac aac ttc cac ccg agt   528
Val Met Pro Thr Asn Leu Tyr Gly Pro His Asp Asn Phe His Pro Ser
                165                 170                 175

aat tcg cat gtg atc cca gca ttg ctg cgt cgc ttc cac gag gcg acg   576
Asn Ser His Val Ile Pro Ala Leu Leu Arg Arg Phe His Glu Ala Thr
            180                 185                 190

gca cag aat gcg ccg gac gtg gtg gta tgg ggc agc ggt aca ccg atg   624
Ala Gln Asn Ala Pro Asp Val Val Val Trp Gly Ser Gly Thr Pro Met
        195                 200                 205

cgc gaa ttt ctg cac gtc gat gat atg gcg gcg gcg agc att cat gtc   672
Arg Glu Phe Leu His Val Asp Asp Met Ala Ala Ala Ser Ile His Val
    210                 215                 220

atg gag ctg gcg cat gaa gtc tgg ctg gag aac acc cag ccg atg ttg   720
Met Glu Leu Ala His Glu Val Trp Leu Glu Asn Thr Gln Pro Met Leu
225                 230                 235                 240

tcg cac att aac gtc ggc acg ggc gtt gac tgc act atc cgc gac gtg   768
Ser His Ile Asn Val Gly Thr Gly Val Asp Cys Thr Ile Arg Asp Val
                245                 250                 255

gcg caa acc atc gcc aaa gtg gtg ggt tac aaa ggc cgg gtg gtt ttt   816
Ala Gln Thr Ile Ala Lys Val Val Gly Tyr Lys Gly Arg Val Val Phe
            260                 265                 270

gat gcc agc aaa ccg gat ggc acg ccg cgc aaa ctg ctg gat gtg acg   864
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Asp Ala Ser Lys Pro Asp Gly Thr Pro Arg Lys Leu Leu Asp Val Thr
        275                 280                 285

cgc ctg cat cag ctt ggc tgg tat cac gaa atc tca ctg gaa gcg ggg   912
Arg Leu His Gln Leu Gly Trp Tyr His Glu Ile Ser Leu Glu Ala Gly
    290                 295                 300

ctt gcc agc act tac cag tgg ttc ctt gag aat caa gac cgc ttt cgg   960
Leu Ala Ser Thr Tyr Gln Trp Phe Leu Glu Asn Gln Asp Arg Phe Arg
305                 310                 315                 320

ggg taa                                                          966
Gly
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             20                  25                  30

Leu Arg Thr Arg Asp Glu Leu Asn Leu Leu Asp Ser Arg Ala Val His
         35                  40                  45

Asp Phe Phe Ala Ser Glu Arg Ile Asp Gln Val Tyr Leu Ala Ala Ala
     50                  55                  60

Lys Val Gly Gly Ile Val Ala Asn Asn Thr Tyr Pro Ala Asp Phe Ile
 65                  70                  75                  80

Tyr Gln Asn Met Met Ile Glu Ser Asn Ile Ile His Ala Ala His Gln
                 85                  90                  95

Asn Asp Val Asn Lys Leu Leu Phe Leu Gly Ser Ser Cys Ile Tyr Pro
            100                 105                 110

Lys Leu Ala Lys Gln Pro Met Ala Glu Ser Glu Leu Leu Gln Gly Thr
        115                 120                 125

Leu Glu Pro Thr Asn Glu Pro Tyr Ala Ile Ala Lys Ile Ala Gly Ile
    130                 135                 140
```

```
Lys Leu Cys Glu Ser Tyr Asn Arg Gln Tyr Gly Arg Asp Tyr Arg Ser
145                 150                 155                 160

Val Met Pro Thr Asn Leu Tyr Gly Pro His Asp Asn Phe His Pro Ser
                165                 170                 175

Asn Ser His Val Ile Pro Ala Leu Leu Arg Arg Phe His Glu Ala Thr
            180                 185                 190

Ala Gln Asn Ala Pro Asp Val Val Val Trp Gly Ser Gly Thr Pro Met
        195                 200                 205

Arg Glu Phe Leu His Val Asp Asp Met Ala Ala Ala Ser Ile His Val
    210                 215                 220

Met Glu Leu Ala His Glu Val Trp Leu Glu Asn Thr Gln Pro Met Leu
225                 230                 235                 240

Ser His Ile Asn Val Gly Thr Gly Val Asp Cys Thr Ile Arg Asp Val
                245                 250                 255

Ala Gln Thr Ile Ala Lys Val Val Gly Tyr Lys Gly Arg Val Val Phe
            260                 265                 270

Asp Ala Ser Lys Pro Asp Gly Thr Pro Arg Lys Leu Leu Asp Val Thr
        275                 280                 285

Arg Leu His Gln Leu Gly Trp Tyr His Glu Ile Ser Leu Glu Ala Gly
    290                 295                 300

Leu Ala Ser Thr Tyr Gln Trp Phe Leu Glu Asn Gln Asp Arg Phe Arg
305                 310                 315                 320

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                Met Gly Glu Pro Gln Gly Ser Met Arg Ile Leu Val
                  1               5                  10

aca ggg ggc tct ggg ctg gta ggc aaa gcc atc cag aag gtg gta gca   158
Thr Gly Gly Ser Gly Leu Val Gly Lys Ala Ile Gln Lys Val Val Ala
          15                  20                  25

gat gga gct gga ctt cct gga gag gac tgg gtg ttt gtc tcc tct aaa   206
Asp Gly Ala Gly Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys
      30                  35                  40

gac gcc gat ctc acg gat aca gca cag acc cgc gcc ctg ttt gag aag   254
Asp Ala Asp Leu Thr Asp Thr Ala Gln Thr Arg Ala Leu Phe Glu Lys
  45                  50                  55                  60

gtc caa ccc aca cac gtc atc cat ctt gct gca atg gtg ggg ggc ctg   302
Val Gln Pro Thr His Val Ile His Leu Ala Ala Met Val Gly Gly Leu
                  65                  70                  75

ttc cgg aat atc aaa tac aat ttg gac ttc tgg agg aaa aac gtg cac   350
Phe Arg Asn Ile Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His
              80                  85                  90

atg aac gac aac gtc ctg cac tcg gcc ttt gag gtg ggg gcc cgc aag   398
Met Asn Asp Asn Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys
          95                  100                 105

gtg gtg tcc tgc ctg tcc acc tgt atc ttc cct gac aag acg acc tac   446
Val Val Ser Cys Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr
      110                 115                 120

ccg ata gat gag acc atg atc cac aat ggg cct ccc cac aac agc aat   494
Pro Ile Asp Glu Thr Met Ile His Asn Gly Pro Pro His Asn Ser Asn
  125                 130                 135                 140

ttt ggg tac tcg tat gcc aag agg atg atc gac gtg cag aac agg gcc   542
Phe Gly Tyr Ser Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala
                  145                 150                 155

tac ttc cag cag tac ggc tgc acc ttc acc gct gtc atc ccc acc aac   590
Tyr Phe Gln Gln Tyr Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn
              160                 165                 170

gtt ttc ggg ccc cac gac aac ttc aac atc gag gat ggc cac gtg ctg   638
Val Phe Gly Pro His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu
          175                 180                 185
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cct ggc ctc atc cac aag gtg cac ctg gcc aag agc agc ggc tcg gcc   686
Pro Gly Leu Ile His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala
    190                 195                 200

ctg acg gtg tgg ggt aca ggg aat ccg cgg agg cag ttc ata tac tcg   734
Leu Thr Val Trp Gly Thr Gly Asn Pro Arg Arg Gln Phe Ile Tyr Ser
205                 210                 215                 220

ctg gac ctg gcc cag ctc ttt atc tgg gtc ctg cgg gag tac aat gaa   782
Leu Asp Leu Ala Gln Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu
                225                 230                 235

gtg gag ccc atc atc ctc tcc gtg ggc gag gaa gat gag gtc tcc atc   830
Val Glu Pro Ile Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile
            240                 245                 250

aag gag gca gcc gag gcg gtg gtg gag gcc atg gac ttc cat ggg gaa   878
Lys Glu Ala Ala Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu
        255                 260                 265

gtc acc ttt gat aca acc aag tcg gat ggg cag ttt aag aag aca gcc   926
Val Thr Phe Asp Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala
    270                 275                 280

agt aac agc aag ctg agg acc tac ctg ccc gac ttc cgg ttc aca ccc   974
Ser Asn Ser Lys Leu Arg Thr Tyr Leu Pro Asp Phe Arg Phe Thr Pro
285                 290                 295                 300

ttc aag cag gcg gtg aag gag acc tgt gct tgg ttc act gac aac tac   1022
Phe Lys Gln Ala Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr
                305                 310                 315

gag cag gcc cgg aag tga agctggaaga caggatcagg tgccagcgga          1070
Glu Gln Ala Arg Lys
            320

ccatcggctg gcagagccca gcggccacca cccgtcaacc ctgccaggag ctgagggcac 1130

cacccagcaa cctgggcctg cattccatcc gctctgcagc cccaagcatc tttccagtgg 1190

ggcccccatt cacgttggtc ctcagggaaa ccagggtccg gggcaggccc ggcgctttgc 1250

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             20                  25                  30

Leu Pro Gly Glu Asp Trp Val Phe Val Ser Ser Lys Asp Ala Asp Leu
         35                  40                  45

Thr Asp Thr Ala Gln Thr Arg Ala Leu Phe Glu Lys Val Gln Pro Thr
     50                  55                  60

His Val Ile His Leu Ala Ala Met Val Gly Gly Leu Phe Arg Asn Ile
 65                  70                  75                  80

Lys Tyr Asn Leu Asp Phe Trp Arg Lys Asn Val His Met Asn Asp Asn
                 85                  90                  95

Val Leu His Ser Ala Phe Glu Val Gly Ala Arg Lys Val Val Ser Cys
            100                 105                 110

Leu Ser Thr Cys Ile Phe Pro Asp Lys Thr Thr Tyr Pro Ile Asp Glu
        115                 120                 125

Thr Met Ile His Asn Gly Pro Pro His Asn Ser Asn Phe Gly Tyr Ser
    130                 135                 140

Tyr Ala Lys Arg Met Ile Asp Val Gln Asn Arg Ala Tyr Phe Gln Gln
145                 150                 155                 160

Tyr Gly Cys Thr Phe Thr Ala Val Ile Pro Thr Asn Val Phe Gly Pro
                165                 170                 175

His Asp Asn Phe Asn Ile Glu Asp Gly His Val Leu Pro Gly Leu Ile
            180                 185                 190

His Lys Val His Leu Ala Lys Ser Ser Gly Ser Ala Leu Thr Val Trp
        195                 200                 205

Gly Thr Gly Asn Pro Arg Arg Gln Phe Ile Tyr Ser Leu Asp Leu Ala
    210                 215                 220

Gln Leu Phe Ile Trp Val Leu Arg Glu Tyr Asn Glu Val Glu Pro Ile
```

225 230 235 240

Ile Leu Ser Val Gly Glu Glu Asp Glu Val Ser Ile Lys Glu Ala Ala
245 250 255

Glu Ala Val Val Glu Ala Met Asp Phe His Gly Glu Val Thr Phe Asp
260 265 270

Thr Thr Lys Ser Asp Gly Gln Phe Lys Lys Thr Ala Ser Asn Ser Lys
275 280 285

Leu Arg Thr Tyr Leu Pro Asp Phe Arg Phe Thr Pro Phe Lys Gln Ala
290 295 300

Val Lys Glu Thr Cys Ala Trp Phe Thr Asp Asn Tyr Glu Gln Ala Arg
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Lys

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Cys Val Gln Leu Leu Gln Asn Gly His Asp Val Ile Ile Leu Asp Asn
20 25 30

ctc tgt aac agt aag cgc agc gta ctg cct gtt atc gag cgt tta ggc 144
Leu Cys Asn Ser Lys Arg Ser Val Leu Pro Val Ile Glu Arg Leu Gly
35 40 45

ggc aaa cat cca acg ttt gtt gaa ggc gat att cgt aac gaa gcg ttg 192
Gly Lys His Pro Thr Phe Val Glu Gly Asp Ile Arg Asn Glu Ala Leu
50 55 60

atg acc gag atc ctg cac gat cac gct atc gac acc gtg atc cac ttc 240
Met Thr Glu Ile Leu His Asp His Ala Ile Asp Thr Val Ile His Phe

```
65                  70                  75                  80

gcc ggg ctg aaa gcc gtg ggc gaa tcg gta caa aaa ccg ctg gaa tat   288
Ala Gly Leu Lys Ala Val Gly Glu Ser Val Gln Lys Pro Leu Glu Tyr
                85                  90                  95

tac gac aac aat gtc aac ggc act ctg cgc ctg att agc gcc atg cgc   336
Tyr Asp Asn Asn Val Asn Gly Thr Leu Arg Leu Ile Ser Ala Met Arg
            100                 105                 110

gcc gct aac gtc aaa aac ttt att ttt agc tcc tcc gcc acc gtt tat   384
Ala Ala Asn Val Lys Asn Phe Ile Phe Ser Ser Ser Ala Thr Val Tyr
        115                 120                 125

ggc gat cag ccc aaa att cca tac gtt gaa agc ttc ccg acc ggc aca   432
Gly Asp Gln Pro Lys Ile Pro Tyr Val Glu Ser Phe Pro Thr Gly Thr
    130                 135                 140

ccg caa agc cct tac ggc aaa agc aag ctg atg gtg gaa cag atc ctc   480
Pro Gln Ser Pro Tyr Gly Lys Ser Lys Leu Met Val Glu Gln Ile Leu
145                 150                 155                 160

acc gat ctg caa aaa gcc cag ccg gac tgg agc att gcc ctg ctg cgc   528
Thr Asp Leu Gln Lys Ala Gln Pro Asp Trp Ser Ile Ala Leu Leu Arg
                165                 170                 175

tac ttc aac ccg gtt ggc gcg cat ccg tcg ggc gat atg ggc gaa gat   576
Tyr Phe Asn Pro Val Gly Ala His Pro Ser Gly Asp Met Gly Glu Asp
            180                 185                 190

ccg caa ggc att ccg aat aac ctg atg cca tac atc gcc cag gtt gct   624
Pro Gln Gly Ile Pro Asn Asn Leu Met Pro Tyr Ile Ala Gln Val Ala
        195                 200                 205

gta ggc cgt cgc gac tcg ctg gcg att ttt ggt aac gat tat ccg acc   672
Val Gly Arg Arg Asp Ser Leu Ala Ile Phe Gly Asn Asp Tyr Pro Thr
    210                 215                 220

gaa gat ggt act ggc gta cgc gat tac atc cac gta atg gat ctg gcg   720
Glu Asp Gly Thr Gly Val Arg Asp Tyr Ile His Val Met Asp Leu Ala
225                 230                 235                 240

gac ggt cac gtc gtg gcg atg gaa aaa ctg gcg aac aag cca ggc gta   768
Asp Gly His Val Val Ala Met Glu Lys Leu Ala Asn Lys Pro Gly Val
                245                 250                 255

cac atc tac aac ctc ggc gct ggc gta ggc aac agc gtg ctg gac gtg   816
His Ile Tyr Asn Leu Gly Ala Gly Val Gly Asn Ser Val Leu Asp Val
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```
            260                 265                 270

gtt aat gcc ttc agc aaa gcc tgc ggc aaa ccg gtt aat tat cat ttt   864
Val Asn Ala Phe Ser Lys Ala Cys Gly Lys Pro Val Asn Tyr His Phe
        275                 280                 285

gca ccg cgt cgc gag ggc gac ctt ccg gcc tac tgg gcg gac gcc agc   912
Ala Pro Arg Arg Glu Gly Asp Leu Pro Ala Tyr Trp Ala Asp Ala Ser
    290                 295                 300

aaa gcc gac cgt gaa ctg aac tgg cgc gta acg cgc aca ctc gat gaa   960
Lys Ala Asp Arg Glu Leu Asn Trp Arg Val Thr Arg Thr Leu Asp Glu
305                 310                 315                 320

atg gcg cag gac acc tgg cac tgg cag tca cgc cat cca cag gga tat  1008
Met Ala Gln Asp Thr Trp His Trp Gln Ser Arg His Pro Gln Gly Tyr
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Pro Asp
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<210> 8
<211> 338
<212> PRT
<213> Escherichia coli

<400> 8

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Cys Val Gln Leu Leu Gln Asn Gly His Asp Val Ile Ile Leu Asp Asn
             20                  25                  30

Leu Cys Asn Ser Lys Arg Ser Val Leu Pro Val Ile Glu Arg Leu Gly
         35                  40                  45

Gly Lys His Pro Thr Phe Val Glu Gly Asp Ile Arg Asn Glu Ala Leu
     50                  55                  60

Met Thr Glu Ile Leu His Asp His Ala Ile Asp Thr Val Ile His Phe
 65                  70                  75                  80

Ala Gly Leu Lys Ala Val Gly Glu Ser Val Gln Lys Pro Leu Glu Tyr
                 85                  90                  95

Tyr Asp Asn Asn Val Asn Gly Thr Leu Arg Leu Ile Ser Ala Met Arg
            100                 105                 110
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Ala Ala Asn Val Lys Asn Phe Ile Phe Ser Ser Ser Ala Thr Val Tyr
        115                 120                 125

Gly Asp Gln Pro Lys Ile Pro Tyr Val Glu Ser Phe Pro Thr Gly Thr
    130                 135                 140

Pro Gln Ser Pro Tyr Gly Lys Ser Lys Leu Met Val Glu Gln Ile Leu
145                 150                 155                 160

Thr Asp Leu Gln Lys Ala Gln Pro Asp Trp Ser Ile Ala Leu Leu Arg
                165                 170                 175

Tyr Phe Asn Pro Val Gly Ala His Pro Ser Gly Asp Met Gly Glu Asp
            180                 185                 190

Pro Gln Gly Ile Pro Asn Asn Leu Met Pro Tyr Ile Ala Gln Val Ala
        195                 200                 205

Val Gly Arg Arg Asp Ser Leu Ala Ile Phe Gly Asn Asp Tyr Pro Thr
    210                 215                 220

Glu Asp Gly Thr Gly Val Arg Asp Tyr Ile His Val Met Asp Leu Ala
225                 230                 235                 240

Asp Gly His Val Val Ala Met Glu Lys Leu Ala Asn Lys Pro Gly Val
                245                 250                 255

His Ile Tyr Asn Leu Gly Ala Gly Val Gly Asn Ser Val Leu Asp Val
            260                 265                 270

Val Asn Ala Phe Ser Lys Ala Cys Gly Lys Pro Val Asn Tyr His Phe
        275                 280                 285

Ala Pro Arg Arg Glu Gly Asp Leu Pro Ala Tyr Trp Ala Asp Ala Ser
    290                 295                 300

Lys Ala Asp Arg Glu Leu Asn Trp Arg Val Thr Arg Thr Leu Asp Glu
305                 310                 315                 320

Met Ala Gln Asp Thr Trp His Trp Gln Ser Arg His Pro Gln Gly Tyr
                325                 330                 335

Pro Asp
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<210> 9
<211> 1047

<212> DNA
<213> Homo sapiens

<220>
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  1               5                  10                  15

cac acg gtg ctg gag ctg ctg gag gct ggc tac ttg cct gtg gtc atc   96
His Thr Val Leu Glu Leu Leu Glu Ala Gly Tyr Leu Pro Val Val Ile
             20                  25                  30

gat aac ttc cat aat gcc ttc cgt gga ggg ggc tcc ctg cct gag agc  144
Asp Asn Phe His Asn Ala Phe Arg Gly Gly Gly Ser Leu Pro Glu Ser
         35                  40                  45

ctg cgg cgg gtc cag gag ctg aca ggc cgc tct gtg gag ttt gag gag  192
Leu Arg Arg Val Gln Glu Leu Thr Gly Arg Ser Val Glu Phe Glu Glu
    50                  55                  60

atg gac att ttg gac cag gga gcc cta cag cgt ctc ttc aaa aag tac  240
Met Asp Ile Leu Asp Gln Gly Ala Leu Gln Arg Leu Phe Lys Lys Tyr
65                  70                  75                  80

agc ttt atg gcg gtc atc cac ttt gcg ggg ctc aag gcc gtg ggc gag  288
Ser Phe Met Ala Val Ile His Phe Ala Gly Leu Lys Ala Val Gly Glu
                85                  90                  95

tcg gtg cag aag cct ctg gat tat tac aga gtt aac ctg acc ggg acc  336
Ser Val Gln Lys Pro Leu Asp Tyr Tyr Arg Val Asn Leu Thr Gly Thr
            100                 105                 110

atc cag ctt ctg gag atc atg aag gcc cac ggg gtg aag aac ctg gtg  384
Ile Gln Leu Leu Glu Ile Met Lys Ala His Gly Val Lys Asn Leu Val
        115                 120                 125

ttc agc agc tca gcc act gtg tac ggg aac ccc cag tac ctg ccc ctt  432
Phe Ser Ser Ser Ala Thr Val Tyr Gly Asn Pro Gln Tyr Leu Pro Leu
    130                 135                 140

gat gag gcc cac ccc acg ggt ggt tgt acc aac cct tac ggc aag tcc  480
Asp Glu Ala His Pro Thr Gly Gly Cys Thr Asn Pro Tyr Gly Lys Ser
145                 150                 155                 160
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aag ttc ttc atc gag gaa atg atc cgg gac ctg tgc cag gca gac aag   528
Lys Phe Phe Ile Glu Glu Met Ile Arg Asp Leu Cys Gln Ala Asp Lys
                165                 170                 175

act tgg aac gta gtg ctg ctg cgc tat ttc aac ccc aca ggt gcc cat   576
Thr Trp Asn Val Val Leu Leu Arg Tyr Phe Asn Pro Thr Gly Ala His
            180                 185                 190

gcc tct ggc tgc att ggt gag gat ccc cag ggc ata ccc aac aac ctc   624
Ala Ser Gly Cys Ile Gly Glu Asp Pro Gln Gly Ile Pro Asn Asn Leu
        195                 200                 205

atg cct tat gtc tcc cag gtg gcg atc ggg cga cgg gag gcc ctg aat   672
Met Pro Tyr Val Ser Gln Val Ala Ile Gly Arg Arg Glu Ala Leu Asn
    210                 215                 220

gtc ttt ggc aat gac tat gac aca gag gat ggc aca ggt gtc cgg gat   720
Val Phe Gly Asn Asp Tyr Asp Thr Glu Asp Gly Thr Gly Val Arg Asp
225                 230                 235                 240

tac atc cat gtc gtg gat ctg gcc aag ggc cac att gca gcc tta agg   768
Tyr Ile His Val Val Asp Leu Ala Lys Gly His Ile Ala Ala Leu Arg
                245                 250                 255

aag ctg aaa gaa cag tgt ggc tgc cgg atc tac aac ctg ggc acg ggc   816
Lys Leu Lys Glu Gln Cys Gly Cys Arg Ile Tyr Asn Leu Gly Thr Gly
            260                 265                 270

aca ggc tat tca gtg ctg cag atg gtc cag gct atg gag aag gcc tct   864
Thr Gly Tyr Ser Val Leu Gln Met Val Gln Ala Met Glu Lys Ala Ser
        275                 280                 285

ggg aag aag atc ccg tac aag gtg gtg gca cgg cgg gaa ggt gat gtg   912
Gly Lys Lys Ile Pro Tyr Lys Val Val Ala Arg Arg Glu Gly Asp Val
    290                 295                 300

gca gcc tgt tac gcc aac ccc agc ctg gcc caa gag gag ctg ggg tgg   960
Ala Ala Cys Tyr Ala Asn Pro Ser Leu Ala Gln Glu Glu Leu Gly Trp
305                 310                 315                 320

aca gca gcc tta ggg ctg gac agg atg tgt gag gat ctc tgg cgc tgg  1008
Thr Ala Ala Leu Gly Leu Asp Arg Met Cys Glu Asp Leu Trp Arg Trp
                325                 330                 335

cag aag cag aat cct tca ggc ttt ggc acg caa gcc tga              1047
Gln Lys Gln Asn Pro Ser Gly Phe Gly Thr Gln Ala
            340                 345
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<211> 348
<212> PRT
<213> Homo sapiens

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His Thr Val Leu Glu Leu Leu Glu Ala Gly Tyr Leu Pro Val Val Ile
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Asp Asn Phe His Asn Ala Phe Arg Gly Gly Gly Ser Leu Pro Glu Ser
35 40 45

Leu Arg Arg Val Gln Glu Leu Thr Gly Arg Ser Val Glu Phe Glu Glu
50 55 60

Met Asp Ile Leu Asp Gln Gly Ala Leu Gln Arg Leu Phe Lys Lys Tyr
65 70 75 80

Ser Phe Met Ala Val Ile His Phe Ala Gly Leu Lys Ala Val Gly Glu
85 90 95

Ser Val Gln Lys Pro Leu Asp Tyr Tyr Arg Val Asn Leu Thr Gly Thr
100 105 110

Ile Gln Leu Leu Glu Ile Met Lys Ala His Gly Val Lys Asn Leu Val
115 120 125

Phe Ser Ser Ser Ala Thr Val Tyr Gly Asn Pro Gln Tyr Leu Pro Leu
130 135 140

Asp Glu Ala His Pro Thr Gly Gly Cys Thr Asn Pro Tyr Gly Lys Ser
145 150 155 160

Lys Phe Phe Ile Glu Glu Met Ile Arg Asp Leu Cys Gln Ala Asp Lys
165 170 175

Thr Trp Asn Val Val Leu Leu Arg Tyr Phe Asn Pro Thr Gly Ala His
180 185 190

Ala Ser Gly Cys Ile Gly Glu Asp Pro Gln Gly Ile Pro Asn Asn Leu
195 200 205

Met Pro Tyr Val Ser Gln Val Ala Ile Gly Arg Arg Glu Ala Leu Asn
210 215 220

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Val Phe Gly Asn Asp Tyr Asp Thr Glu Asp Gly Thr Gly Val Arg Asp
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Tyr Ile His Val Val Asp Leu Ala Lys Gly His Ile Ala Ala Leu Arg
                245                 250                 255

Lys Leu Lys Glu Gln Cys Gly Cys Arg Ile Tyr Asn Leu Gly Thr Gly
            260                 265                 270

Thr Gly Tyr Ser Val Leu Gln Met Val Gln Ala Met Glu Lys Ala Ser
        275                 280                 285

Gly Lys Lys Ile Pro Tyr Lys Val Val Ala Arg Arg Glu Gly Asp Val
    290                 295                 300

Ala Ala Cys Tyr Ala Asn Pro Ser Leu Ala Gln Glu Glu Leu Gly Trp
305                 310                 315                 320

Thr Ala Ala Leu Gly Leu Asp Arg Met Cys Glu Asp Leu Trp Arg Trp
                325                 330                 335

Gln Lys Gln Asn Pro Ser Gly Phe Gly Thr Gln Ala
            340                 345
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<212> PRT
<213> Artificial Sequence

<220>
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<400> 11

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40                  45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Xaa Xaa Ala Xaa Xaa Xaa
    50                  55                  60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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Asn Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

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100 105 110

Xaa Xaa Xaa Pro Xaa Xaa Glu Xaa Xaa Xaa Xaa Xaa Gly Xaa Xaa Xaa
115 120 125

Xaa Xaa Xaa Xaa Xaa Tyr Xaa Xaa Xaa Lys Xaa Xaa Xaa Xaa Xaa Xaa
130 135 140

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
145 150 155 160

Xaa Xaa Asn Xaa Xaa Gly Xaa His Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
165 170 175

Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
180 185 190

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Xaa Gly Xaa Xaa Xaa Arg Xaa
195 200 205

Xaa Xaa Xaa Xaa Xaa Asp Xaa Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
210 215 220

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
225 230 235 240

Xaa Xaa Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
245 250 255

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Phe Xaa Xaa
260 265 270

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
275 280 285

Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
290 295 300

Xaa Thr Xaa Xaa Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
305 310 315

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ctggagtcga attcatgagt aaacaacgag 30

<210> 15
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<400> 15
aactgcagtt acccccgaaa gcggtcttga ttc 33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11576

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C12P 19/00, 17/04; C12N 1/12, 1/20, 5/00, 5/04
US CL :435/72, 126, 252.1, 252.3, 410, 419
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/72, 126, 252.1, 252.3, 410, 419

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, MEDLINE, EMBASE, BIOSIS, SCISEARCH, BIOTECHDS, NTIS, WPIDS, HCAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 85/01745 A1 (KRAFT, INC.) 25 April 1985 (23.04.85), see the entire document specially ages 4-7.	1-72
Y	NIKISHIMI et al. Occupance in Yeast of L-Galactonolactone Oxidase which is similar to a key enzyme for Ascorbic Acid biosynthesis in animals, L-Gulonolactone Oxidase. Arch. Biocem. Biophys. December 1978, Vol. 191, No. 2, pages 479-486, see the entire article, specially abstract and introduction sections.	1-72
A,P	WO 99/33995 A1 (ASCORBX LIMITED) 08 July 1999 (08.07.99), see the entire article.	1-72

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
23 AUGUST 1999

Date of mailing of the international search report
22 OCT 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
MARYAM MONSHIPOURI
JOYCE BRIDGERS
PAR[illegible] SPECIALIST
Chemical Matrix
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11576

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11576

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This international Preliminary Examining Authority has found 2 inventions claimed in the International application covered by the claims indicated below:

Group I, claims 1-59 and 71, drawn to a method of producing ascorbic acid or esters thereof in a microorganism comprising culturing a microorganism having a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase etc. as well as a microorganism genetically modified for producing ascorbic acid.

Group II, claims 60-70 and 72, drawn to a plant for producing ascorbic acid or esters thereof, wherein said plant has a genetic modification to increase the action of an enzyme selected from the group consisting of hexokinase, glucose phosphate isomerase etc.

The inventions listed as Groups I-II do not relate to a single inventive concept because they are considered to be two different categories of invention and are not drawn to combination of categories (i.e. categories 1-5), specified in 37 CFR section 1.475(b).